

ANNUAL REVIEW OF PHYSIOLOGY



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# ANNUAL REVIEW OF PHYSIOLOGY

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## PREFACE

Imagine the physiologist of tomorrow seated at his desk, planning the next step in his research program. An immediate question: what is the latest from others studying the same problem? He reaches for his information retriever, dials the code numbers for the effect of estrogen on the glycogen content of the uterine tube of the fetal guinea pig, gives the computer (located perhaps in Wichita) a few seconds to search its store of data, then watches his desk television screen as it displays, one after another, abstracts of every paper ever published containing this information. He presses a button now and then to obtain instantaneous copies of items which look promising. His literature search is done.

At his elbow is a shelf. Does it contain the familiar maroon of the accumulated volumes of the *Annual Review of Physiology*? Or are they long a thing of the past, along with letter-press publication of the journals, the snail-paced mountainous indices and the miles of microfilm too awkward to consult?

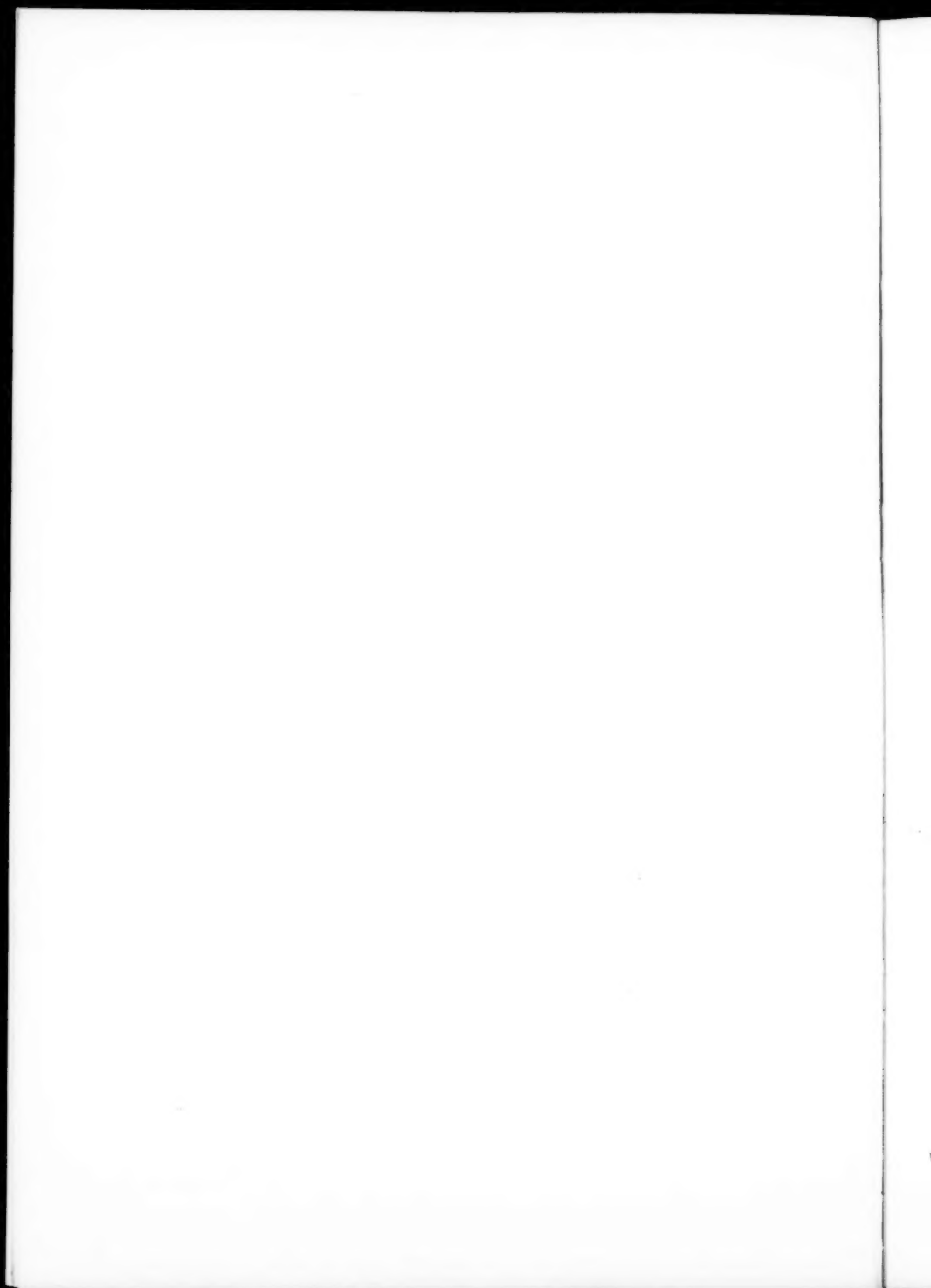
We think not. The *Review* will still be there, and with a more clear-cut function than it now possesses. It will no longer even try to help the specialist find new material in his field, but will continue to do something beyond the foreseeable ability of computers: to sift the excellent from the merely good, to recognize and evaluate new trends in physiological thought, to warn against blind fashion, and to keep the enduring questions concerning the function of living matter firmly before us. To do these things has always been one of our purposes. We welcome the pressures of the future which will make them our sole purpose.

Meanwhile we carry on, again with deep appreciation of and gratitude to our authors for their building of this volume. Kind fortune has permitted us to keep the Assistant Editor, Joann Huddleston, at the controls. The George Banta Company, our printers, continue to please us with their work.

R.S.A.  
J.M.B.  
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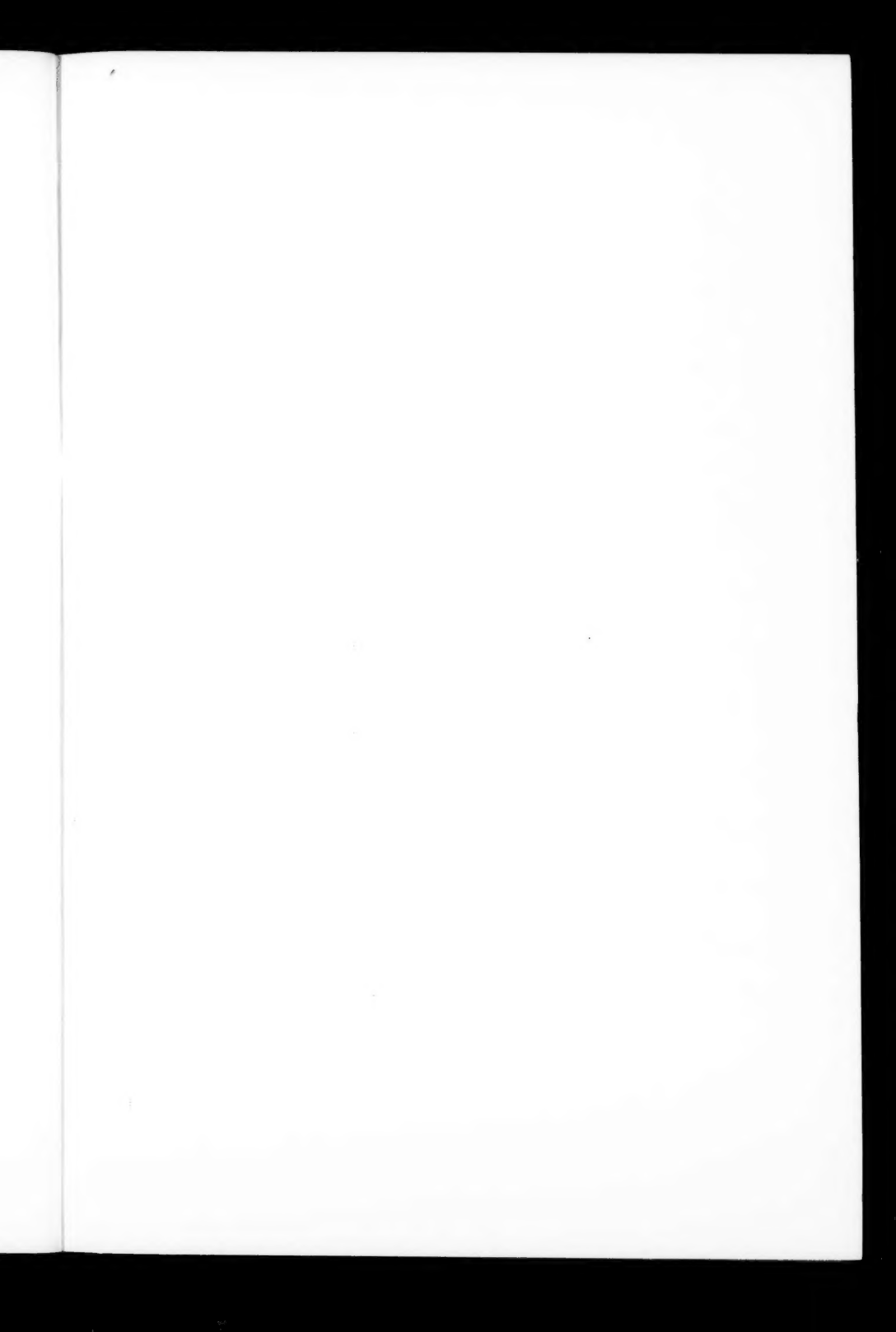
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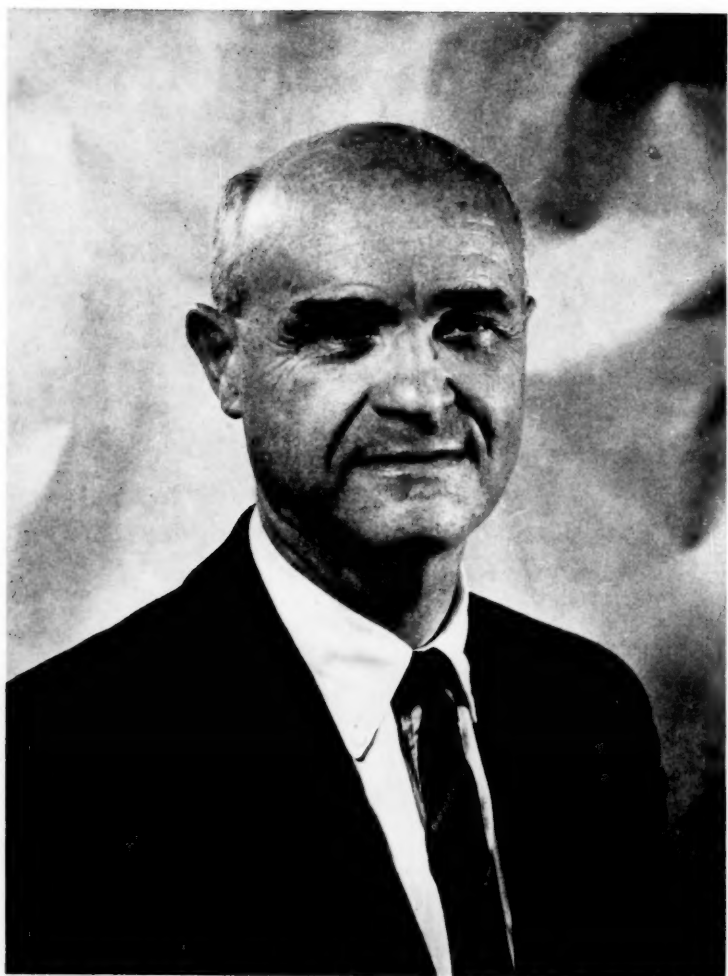
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CARL F. SCHMIDT, M.D.

## PHARMACOLOGY IN A CHANGING WORLD

BY CARL F. SCHMIDT

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In one who for many years has been called on annually to bid God-speed to a class of medical students, an invitation to contribute the prefatory chapter to a volume of the *Annual Review of Physiology* arouses familiar emotions. The occasion clearly calls for something out of the ordinary; yet from previous experience one knows that after the event he will look back on it unhappily, wondering why, with all the words he might have used, he chose those particular ones. He cannot take the task lightly, partly because now and then he learns from former students that words which he had long forgotten were thought to have been of value to them at a critical stage of their careers. He knows that the credit for such an impression belongs, not to any words of his, but to coincidental first successes in the performance of adult tasks by young people of such caliber that they would do well in anything they undertook. Yet he realizes that, if some of these in retrospect believe their subsequent success to have been even slightly influenced by his efforts, they must indeed have been eagerly searching for guidance in a confusing world. So he tries again and again to find the winged words and golden thoughts that had eluded him in the past until at last he hands the task on to his successor, realizing that he has been seeking something that will only be found with the Lost Chord.

The present assignment differs from this long-familiar one in that the audience is more numerous, diverse, and sophisticated, and there are no direct assurances from previous listeners that earlier efforts have served a useful purpose. The latter presumably is compensated by the fact that the Editorial Board continues to dedicate precious space in these volumes to such an undertaking. The former intensifies the desire at least to find a theme appropriate to the occasion.

In perusing the prefatory chapters previously contributed by my contemporaries, I find the term "transitional period" frequently applied to the years of our activities. If this is true of physiology as a whole, it certainly is no less true of pharmacology. The author of the prefatory chapter to the preceding volume (1) discussed his experiences during a transitional period in medicine, which he entered by way of the physiology laboratory. It will be interesting to me to attempt a corresponding review of my own experiences in pharmacology during the same period. My justification for doing this in the *Annual Review of Physiology* is that I have always regarded pharmacology and physiology as sister sciences sharing the same basic interests and employing the same methods.

A discussion of transitions in pharmacology is particularly timely be-

cause this year will mark two events that depict the passage of the science into a new phase. One is the appearance of Volume I of *The Annual Review of Pharmacology*. The other is the First International Pharmacological Meeting. The one is scheduled for early summer, the other for August, both in 1961. The latter is the first official international meeting ever to be arranged by pharmacologists for their own purposes.

I cannot speak for the *Annual Review of Pharmacology*, but I know that the First Pharmacological Meeting actually will find pharmacology, not in a state of new and complete independence, but in a position in which it began over a century ago and in the rôle which made it one of the most intellectually stimulating of all sciences. The theme of this meeting is to be the Mode of Action of Drugs, a problem which enlisted the attention of Claude Bernard (2) before 1860 and which was the main incentive for the foundation of modern pharmacology by Rudolf Buchheim (3). Bernard's interest in drugs lay in their ability to serve as "*scalpels chimiques*" for dissecting out physiological mechanisms and thus for making important additions to fundamental knowledge. Buchheim was primarily concerned with learning the facts about what drugs can and cannot accomplish and was aiming directly at better orientation of the physician in his use of drugs at the bedside. Buchheim's illustrious pupil Schmiedeberg, who was called upon to organize the world's first Laboratory of Pharmacology in Strassburg just after the Franco-Prussian War (4), combined both these concepts of the rôle of pharmacology into a discipline so exciting that it attracted a group of young men of extraordinary brilliance. A partial list of these includes such names as Dale, Richards, Cushny, Abel, Wallace, Dixon, Meyer, Gottlieb, Magnus, Trendelenberg, Ehrlich, Fühner, Heubner, and Fournau. Men such as these would have been welcome in any field and would have made it great. They chose pharmacology because at the time it was the most intellectually stimulating of the medical sciences.

The time was the first decade of the twentieth century, when the Western Hemisphere was just beginning to establish chairs of pharmacology. Epinephrine (adrenaline) had been isolated, chemically identified, and recently synthesized. Tyramine was found to be related chemically and physiologically to epinephrine, and the concept of sympathomimetic amines had just been propounded (5). The similarity between the effects of adrenaline and those of stimulation of the sympathetic nervous system had been noted, and Elliott (6), then a student at Cambridge, made the proposal that sympathetic nerve impulses might act by liberating this chemical agent. Dixon (7) was making a similar claim for muscarine (the actions of which were discovered by Schmiedeberg some years before) as a parasympathetic transmitter. Langley was using nicotine to map out the autonomic nervous system (8), and Cushny had recently shown (9) that the long-familiar pulsus irregularis perpetuus is referable to atrial fibrillation, which he could produce in experimental animals by electrical stimulation. The chemical in-

dusdry was turning out considerable numbers of new compounds, and some of these (acetanilid, acetophentidine, antipyrine, barbiturates) were finding permanent places in therapeutics. Meyer, Overton, Traube, Lillie, Verworn, and others were diligently searching for a general theory of narcosis (10). Perhaps most exciting of all was Ehrlich's undertaking to create specific chemotherapeutic agents according to biological and chemical theory (11).

These and other scintillating events were transpiring in the area previously assigned to *materia medica*, which hitherto had been the least progressive of all medical disciplines. It was also the last to succumb to the invasion of traditional medicine by the scientific method. There had been a long period of revulsion against the injudicious therapeutic practices of preceding generations. Clark (12) cites some of these and quotes the comment "*il est mort guéri*" which sometimes appeared at the end of case histories. According to Buchheim (13), the man who was called pharmacologist in the old days often was assigned the task of performing autopsies, both because he was not as busy or as important as his colleagues and because the major purpose of the autopsy was to convince the family that the patient died of the disease, not of the drugs. During this period, research in *materia medica* had been aimed at finding mixtures and manipulations which would bring out the virtues of a drug and attenuate its shortcomings—a tradition which still survives in the terms "adjuvant" and "corrective" in prescriptions. According to Schmiedeberg (3), Hahnemann believed that both these aims could be achieved by simple dilution, which was one of the main features in his Homeopathic Doctrine.

The revulsion against therapy was accelerated by the emergence of pathology as the accepted route to success in academic, scientific medicine. Pathology took over the autopsy service and made it do much more than protect unsuccessful therapists against legal action. Clark (12) quotes the following from the leader of the rise of modern pathology, Rudolf Virchow: "Therapy is in an empirical stage cared for by practical doctors and clinicians and it is by means of a combination with physiology that it must rise to be a science, which today it is not."

Virchow's advice actually was even then being followed by Buchheim and his colleagues. But the leaders of the transformation of medicine from unverified tradition to a science were, with few exceptions, trained in pathology until the changes outlined by McLean (1) began about 1920. It is scarcely surprising that men whose viewpoint on the possibility of favorably influencing the course of a disease was derived from the study of lost therapeutic battles in the autopsy room should take a dim view on drug-giving.

The preponderant motives of the brilliant group who, just one generation ago, were called upon to become the first professors of pharmacology all over the world, clearly did not include the prospect of material advantages, scientific prestige, or immediate benefit to humanity. The chairs of pharmacology during the first two decades of this century were poorly supported, and

the newly appointed professors in many if not most cases were viewed with condescension by their colleagues in the older scientific disciplines, with indifference, hostility, or contempt by the clinicians. The immediate reason for adding them to the medical faculties seems to have been a determination on the part of a few energetic faculty members and alumni to bring the institution abreast of this latest trend in medical education. Sometimes the newcomer supplanted a professor of pharmacology and *materia medica* whose approach had been the traditional one of *materia medica*. In such cases one of the immediate results was a series of complaints from the clinical faculties that the students no longer were being taught anything about drugs, and from the students that they were at a disadvantage in competition with their contemporaries in other institutions because they no longer were given lists of time-honored prescriptions.

This was the situation at Pennsylvania when I entered the medical school in 1914. A. N. Richards had been appointed Professor of Pharmacology in 1910, and after a term in Schmiedeberg's laboratory he began to organize a course along the new lines. By the time I took this course in 1915 he had established a reputation as a splendid teacher with a fetish for perfection. His insistence on valid evidence and his emphasis on critical examination of the method made a deep impression on his students, including myself. When I was offered an instructorship in his department at the close of my internship in 1919, I was glad to accept.

As I look back, I realize that this was near the end of the era which Schmiedeberg (3) called the Negative Phase of Pharmacology. This period began with the revulsion of the medical profession and the public against prevailing practices of drug-giving, was accelerated by the therapeutic nihilism resulting from the pathological orientation of the leaders of the rising science of medicine, and was climaxed by the accomplishments of the new pharmacologists who tested the available drugs and in many cases found them wanting. Apart from Ehrlich's triumph with the arsphenamines (which many other workers unsuccessfully tried to duplicate with other metals and in other types of infection) and tissue-sparing antiseptics such as Dakin's solution, the organic chloramines, and the organic mercurials, there had been no notable additions to the therapeutic armamentarium for more than ten years. The new generation of pharmacologists had aroused serious doubts about the real value of many widely used drugs but they had not yet provided anything better. These negative findings were appreciated by scientifically minded clinicians, who were thus enabled to avoid deceiving themselves even when they thought it proper to go on deceiving their patients, but the challenge which kept the pharmacologists at work under often discouraging conditions was the one which had first attracted Bernard, Buchheim, and Schmiedeberg, viz. the explanation of how and why drugs do what they do. Nowhere, as far as I know, was there any suggestion that the pharmacologist was expected to do anything but learn the facts about fundamental mechanisms. As a matter of fact, in so doing he was providing

two essentials for the next phase, viz. the methodology for studying new drugs, and an attraction to good young people to enter this fascinating field.

Shortly after my entrance into pharmacology in 1919 began a Positive Phase, in which important new drugs were introduced in rapid succession. The first of these was insulin. Then came ephedrine, important not so much in itself, but rather as a trigger for intensive efforts toward the synthesis and testing of drugs affecting the autonomic nervous system. One of the major reasons for this outcome was that a single drug company, more enterprising than its competitors, sought to secure an advantage over them by cornering the Chinese market on Ma Huang, the vegetable source of ephedrine. As a result the competitors were driven to resort to synthesis of compounds related to ephedrine, and a large variety of these were made and tested. One company found a product that sold so well as to enable them to finance a research organization of their own, which subsequently became quite large and now is very active and productive. Others came up with a variety of new compounds with a spectrum of activities wide enough to permit choice of the agent having a minimum of undesired features for almost any situation. Perhaps all these things would have happened if the natural source of ephedrine had been shared among the manufacturers, but they certainly were accelerated by the attempt of one of them to gain a monopoly.

Then came such events as the mercurial diuretics (intended originally as antiluetics of the arspenamine type), the nonnarcotic anticonvulsants, the synthetic adrenergic and ganglionic blocking agents, standardized curare and synthetic curariform agents, the sex and adrenal steroids and their derivatives, the clinically useful anticoagulants, the antihistaminics, and finally the sulfonamides and antibiotics. The newer anesthetics, the synthetic analgesics, and the muscle relaxants were utilized by new groups of medical anesthesiologists in an enterprise that was stimulated and nurtured by pharmacology. More recently have come the tranquilizers, psychic energizers and hallucinogens, and the orally effective potent diuretics.

When I entered pharmacology in 1919, the physician who went through life depending entirely on the drugs used by his teachers was not doing much harm, either by omission or commission. Three years later the situation had already changed with the introduction of insulin. Like many of my contemporaries, I saw some instances of the early misuse of this drug by practitioners who had not bothered to familiarize themselves with its capacities for doing harm. Similar episodes subsequently arose with the potent diuretics, the sulfonamides, the antibiotics, the intravenous anesthetics, and many others. These new drugs were not like the tonics, alteratives, emollients, demulcents and derivatives of the old days, which might or might not do any good but were unlikely to do harm. They were potent agents and they usually were given in dosages intended to build up and maintain an effective concentration in the body. The physician who used them had to know something about their capacities for harm as well as good, and for this the first step was an awareness of the questions he should have asked and answered before

he went ahead. This is where a modern course in pharmacology enters the picture as the only way of preparing a medical student for life in a constantly changing scene.

For change it certainly will. According to Beckman (14), an average of 400 new prescription items now is being introduced in this country every year, and some fifty of these are new chemical entities. Another source (15) places the total at 3000 in the last ten years and estimates their average life at three to five years. The recent volumes of the *U. S. Pharmacopoeia* are no larger than their predecessors, and the newest (XVI) is actually a little shorter than XV. Obviously the new agents are replacing an equal number of older ones, and the statistics for life expectancy, mortality, and morbidity, as well as the obviously greater usefulness and comfort conferred by some of the new drugs (antidiabetic, antihistaminic, antiarthritic, antiepileptic, tranquilizing, antihypertensive, etc.), all indicate that the public is better off in this respect than ever before.

Where does pharmacology fit into this picture? This question can be directed into the past, the present, and the future. It is now a far cry from 1919, when my accession to the Department of Pharmacology at Pennsylvania increased its numerical strength by fifty per cent, i.e. from two to three. Richards had already started his investigations on kidney function, which were undertaken for the purpose of enabling him to give his students a better explanation for the diuretic effects of caffeine than was then possible. He and Cecil Drinker (while a medical student) had made a perfusion pump which could duplicate the normal pulse pattern and, on perfusing a rabbit kidney with it, had found that the addition of a minute amount of epinephrine produced: (a) rise in perfusion pressure which, since the pump output was constant, indicated vasoconstriction; (b) swelling of the oncometer-enclosed kidney, which indicated vasodilation; and (c) diuresis, which suggested increased glomerular filtration. The best explanation Richards could find for this strange state of affairs was a selective constriction (by epinephrine) of the efferent glomerular vessels, and he set himself and his associates the problem of finding an experimental means for testing this hypothesis, which then was completely new. While perusing Cushny's then-recent monograph (16), we happened to see an inconspicuous allusion to some experiments by Ghiron (17), who reported that he had been able to make microscopic observations of the glomerular circulation in the kidney of a living mouse. After unsuccessful attempts at duplicating these observations, we turned to the flat kidney of the frog, and here we found the preparation Richards had been seeking. Actually it was only after several years of extraordinarily productive work that the preparation was used to determine whether the original hypothesis was valid or not; the answer was affirmative (18).

The next direction of the experiments was determined by a suggestion by Wearn (19) that it might be possible to withdraw glomerular fluid directly from Bowman's capsule by an adaptation of the microdissection technic then being developed by Robert Chambers. These experiments yielded the



first unambiguous evidence ever secured on the nature of kidney function, for the glomerular fluid contained a chloride and a reducing sugar while the bladder urine contained neither (19). Obviously these normal constituents of the blood had left the circulation to enter the glomerular space and had been reabsorbed somewhere between the glomerulus and the bladder. The result was regarded as strong confirmation of the filtration-reabsorption concept of Carl Ludwig. Quantitative studies on other normal blood constituents subsequently indicated that the glomerular fluid is essentially a colloid-free ultrafiltrate of the blood plasma (20). The finding that this is true of easily measurable creatinine furnished the basis for now-familiar clearance studies and thus for the entire mass of modern renal physiology. I like to recall that it all began out of an attempt to improve the teaching of medical students. It seems to me that a young physiologist or pharmacologist who seeks to advance more rapidly by avoiding teaching and concentrating on research is missing a most powerful incentive to get his thoughts arranged in proper perspective.

The work on frogs came at a time when Richards had secured a little money from a friend to enable him to pay for some animals and to buy some needed supplies (including hirudin, the only anticoagulant then available other than citrate and oxalate). Shortly afterward I went to China, and when I came back in 1924 the department was a beehive of activity on the kidney project. Richards had secured support for this from several foundations and the period of poverty was over. He urged me not to jump on the renal bandwagon, but to work on my own problems. I therefore returned to my first interest, which involved an explanation of certain effects of morphine and other drugs on respiration (21). Actually I had never forgotten this problem, and my first days in Peking were spent on trying to isolate hirudin from the heads of Chinese leeches so that I might get on with my plan of measuring cerebral blood flow in relation to respiration. I had expected that my first year in China would be spent in working with Bernard E. Read, the head of the Department of Pharmacology; and for the second year Reid Hunt, Professor of Pharmacology at Harvard, was to be present as visiting professor. What actually happened, however, was that Dr. and Mrs. Hunt arrived a few weeks after I did and a year earlier than Read and I had expected. Read thereupon made a rapid readjustment and left for his sabbatical a year earlier than he had planned. Before he left he outlined his plans for studying Chinese drugs, gave me a list of supposedly important ones, and urged me to have a try at investigating them.

Some of my experiences in this connection have been recorded elsewhere (22). If it had not been for a series of coincidences that led us to ephedrine (my writing to Chen before he came to Peking telling him of my unpromising experiences with Chinese drugs, his telling something of this to an uncle at a family reunion in Shanghai and getting a recommendation to look into Ma Huang, and our trying a hasty aqueous extract of this drug in a preparation for a student experiment), my two years in Peking would have been



highly interesting but scientifically unrewarding. Ephedrine however brought these years from the Negative to the Positive Phase.

By 1925 money was becoming available for research work and we were able to afford occasional cross-circulation and perfusion experiments. Solid heparin had appeared, but it was so expensive (or our fund so small) that we had to resort to repeated bleedings, defibrinations, and reinjections to make it cover as many experiments as possible. We were able to buy the glass parts of some new Van Slyke-Neill blood-gas analyzers, but we had to mount them ourselves and we did our own analyses after the experiment had ended, sometimes spending the entire night on this after a day of complex dissection and experimentation. Apart from an interlude devoted to addiction to morphine [a study undertaken out of a sense of public responsibility but surprisingly interesting from the scientific viewpoint (23)], my work dealt with the interrelations among circulation and respiration which had been revealed by my first independent efforts (21). The results led me to a concept similar to that popularized by Gesell (24), but actually advanced many years earlier by Rosenthal (25) and subsequently by Winterstein (26) and Pearce (27), viz. that the chemical control of breathing depends on the concentration of stimulant chemical material in the cells of the center, and since these are continuously producing such agents by their own metabolism, their activity can be modified by changes in their blood supply. This was the origin of my interest in the cerebral circulation.

The reports by Heymans and his associates (28) on the respiratory effects of reflexes from the carotids and aorta directed my interests into this area and subsequently toward other reflexes. The results forced a reevaluation of the interplay between chemical and reflex factors in respiratory control, a process which is still going on. Similar processes are now beginning in circulatory control (29, 30).

With World War II the days of financial stringency definitely ended. One of the pleasantest episodes of my career was the opportunity afforded by our activities in aviation research to create a climate in which Kety (31) could conceive and develop his method for measuring cerebral blood flow and metabolism in man, and in which means for testing the hypothesis concerning respiratory control were immediately available. It is noteworthy that this situation, like that which brought about Richards' work on kidney function, was the result of a pervading desire for a better understanding of a familiar action of a well-known drug (21).

Schmiedeberg died in 1921. If he and his teacher Buchheim could see the changes in the therapeutic scene in the subsequent forty years, they would have good cause for gratification with the quality of the seed they planted. For all the important drug discoveries during this period were made in research laboratories dedicated to the task on which they and their pupils had worked, viz. to study the actions of drugs by any or all methods that seemed appropriate. They could not have foreseen the rapid increase in financial support of medical research in this country that followed the ex-

ample set by John D. Rockefeller, Sr. just before 1920. Nor could they have anticipated the expansion of the chemical industry and the rapid growth of research among the drug manufacturers. But they would have no cause for surprise at the dramatic results of providing more opportunities for good people to develop and test their ideas in laboratories. Buchheim had this to say in 1857 (3): "Wer imstande ist den schwierigen Teil, die Fragestellung, gut auszuführen, wird mit Leichtigkeit die für seine Untersuchungen geeigneten Methoden auffinden können." He and his pupil Schmiedeberg might smile a little at the recent rediscovery of the importance of new questions to be fed into our modern research machine.

The unprecedented and uninterrupted series of discoveries of new drugs, however, has caused a drastic change in the place of pharmacology among its sister sciences. Its original twofold aim of adding to basic scientific knowledge by using drugs as physiological reagents, and of orienting physicians on the proper uses of their drugs at the bedside, now has been supplemented by a third, viz. to discover and evaluate new agents of therapeutic value. Actually this assignment was familiar even in Buchheim's day (3), but the agents to be tested were so few and infrequent that such activities could be taken more or less in stride with the other functions of the laboratories. Now, however, this certainly is no longer the case. The 400 new drug items introduced every year, and the associated continuous turnover of drugs alluded to above (page 6), represent only the survivors of a much larger number of preparations that had to be tested. This has come to be much more than an appendage of pharmacology.

Such developments have obscured in a golden glow of practical accomplishments the traditional objective of pharmacology, which was to provide facilities and intellectual climates in which gifted young people would want to work and would be encouraged to follow up their ideas by any suitable method. It has always been the prerogative of academic departments of pharmacology to find these people and to prepare them for their careers, and I know of no plan for changing this situation in the future. But in these days of emphasis on research, of mutterings of discontent over "teaching loads", and of freely available fellowships for young people who, under the caption of "graduate training program", can be made to perform tasks which seldom are of the caliber which once made pharmacology exciting, it is becoming increasingly difficult for the director of such a laboratory to have a clear objective at which to aim his policies.

It seems to me that his best objective is to attract into pharmacology a fair share of the young people who are going to be the leaders of the medical sciences in the future. There is now a large and well-supported program for promoting graduate study in the individual medical sciences (including pharmacology), but I wonder how many of the best candidates are going to choose pharmacology rather than biochemistry or physiology. Many science teachers in high schools and colleges have done work in the latter disciplines and are properly enthusiastic over them, but I have yet to hear of one who

is able to speak for the intellectual rewards of pharmacology. These are comprehensible only after a student knows enough about the other medical sciences to appreciate the possibilities of the interdisciplinary approach of pharmacology.

It seems to me that the better place to search for the leaders of pharmacology of the future is among the medical students. These already are a carefully selected, sophisticated group, and if one can attract and hold their respect (by making one's teaching as interesting and challenging as possible), one has at least made a start. In the past we used to measure the success of our teaching program by the number and caliber of the young medical graduates who sought to work in the department. The development of the residency training program has drastically altered this situation, for a young man offered a residency during his internship must come to a decision while close to the attractions of the clinic but remote from the laboratory disciplines. He can cross over subsequently from clinic to laboratory, but the reverse step is seldom possible. We once were able to offer him the enticement of an intellectually stimulating experience in teaching and research, but the modern clinical departments have come to be at least our equal in this respect, and they have the added attraction that the young man remains within the clinical hierarchy and is working toward his specialty certification. Thus the clinical departments hold nearly all the winning cards in the competition for the best young medical men, and this means to me that they are going to have the leaders of the medical sciences of the future unless something is done to alter the present arrangement.

I wish to make it as clear as possible that I do not regard a medical training and degree as essential for success in pharmacology or any other medical science. Such a claim would be ridiculous, not only because the medical course is too rigid, too brief, too shallow, and too diversified to be anything but a preparation for further education, but also because the example of men like Richards, Fenn, Bard, Bronk, and many others shows that distinguished careers are not appreciably hampered by the lack of medical training.

We clearly are entering an era of salaried positions for all connected with the medical sciences, clinical and preclinical. The disparity in salary between the two groups already is narrowing and it may narrow further if the presently-rumored governmental support for medical education materializes.

The basic medical sciences have a greater attraction than ever before for the better young medical graduates, and clinical departments in major medical centers are becoming complete, self-contained medical schools. This is one of the early results of the attraction into these departments, by means of the residency training programs, of the young physicians who once turned to the basic science departments for a year or two of research and teaching before they settled down to clinical careers. Such young people provided an annual transfusion of eager young blood for a department such as ours, and now and then (often enough for our limited resources) one of them would

decide to remain in pharmacology. Since the advent of the residency programs, the young physicians get their research and teaching experience in basic science within the framework of the clinical departments, which now are retaining the strengths they once shared with us.

A department such as ours then may: (a) seek some arrangement which will again enable it to secure a regular rotation of the best young physicians; (b) develop some means for recruiting nonmedical personnel of a caliber equal to that of the better medical students, whom it is going to teach; (c) reconcile itself to medical graduates who could not obtain good residencies and to applicants for graduate training who could not enter medical school; or (d) recast its objectives and activities to fit the new order of things, i.e., allow the clinical departments to take over the most interesting and most exciting features in modern medicine.

I have listed these in the order of their apparent desirability. It is unlikely that (a) can be accomplished by any but the most forceful intervention, for the whole structure of a modern university hospital has come to depend on this source of excellently trained, highly motivated, and poorly paid young men. But we are dealing here with a matter that transcends the convenience of hospital administrators and the ambitions of department heads inclined toward empire building. The real question is the best allocation of the young people who are going to be the leaders of the future. If our entire residency training program were critically reexamined with the view of making the best possible use of the precious young human resources with which it deals, the basic medical sciences might be greatly strengthened.

It is too early to tell what will eventually happen to these departments when the process of development of independent clinical units described by McLean (1) runs its allotted course and the clinical teachers regard themselves as fully competent to teach all the basic science the students need to know. They may be quite right in this, but the problem will come when the goose that laid the golden eggs is to be replaced. Alternative (b) would be ideal if these people could be found in sufficient numbers and with sufficient regularity to make this a dependable source of desirable young personnel. As noted above, pharmacology is at a disadvantage here because the interdisciplinary features that made it attractive to brilliant young men in the past cannot be appreciated until the other sciences have been thoroughly sampled.

The recent and current trend toward a biochemical approach to pharmacology is another manifestation of this long-familiar phenomenon. There can be no doubt about the possibilities of this approach. But it should be recalled that the first entry into pharmacology by Buchheim and his associates was essentially chemical. One of his colleagues (Carl Schmidt by name) was responsible for discovering the free acid of the gastric juice, the presence of chitin in the shells of insects, and the partition of sodium and potassium between red blood cells and plasma (3). Schmiedeberg quite early in his career demonstrated the formation of urea from ammonia in the liver (4).

At that time studies such as these were regarded as part of physiology, not as a separate and competitive discipline. Before Schmiedeberg occupied his permanent chair in Strasburg, he went to round out his repertoire for pharmacology by a term in Ludwig's laboratory of physiology. Richards was trained in biochemistry and was sufficiently esteemed to be asked to edit the *Journal of Biological Chemistry* quite early in his career, but before he settled down he went to Schmiedeberg to learn physiological technics. The accomplishments for which both these men are best known are a combination of chemistry and physiology, and a similar statement can be made about Dale, Meyer, Abel, Ehrlich, and many other great pharmacologists of the past.

For as I see it, the strength of pharmacology has always lain in its broad scope and diversity. It is no longer possible for the director of a laboratory of pharmacology to become a master of all the experimental technics available in all the biological sciences, but he can at least keep his department from specializing in one particular approach, thereby becoming only another department of physiology or biochemistry or microbiology. The distinctive feature of pharmacology is not that it has any methodology peculiar to itself, but that it is ready to use any or all methods to elucidate the mode of action of chemical substances on living systems. The more types of activity there are in a department of pharmacology, the better are its prospects of living up to the traditions of Buchheim, Schmiedeberg, Dale, Richards, Cushny, Abel, and their counterparts. It is not necessary, or even desirable, to go any further than to create a climate to which some of the keenest young minds will be attracted as they were in the past. If this is done, pharmacology will be well served in the future. If not, it probably will be reduced to service functions such as screening programs, toxicity studies, and bioassays, and the other sciences will take over its intellectual appeal.

There are signs of a determined effort to recapture some of the formerly intimate relationships between physiology and biochemistry, at least at the international level. A liaison committee between the International Union of Physiological Sciences (IUPS) and the International Union of Biochemists (IUB) was appointed in 1959 for the purpose of arranging a series of joint programs between the two disciplines. The First International Meeting of Pharmacologists mentioned on page 2 is being held under the auspices of the IUPS, through a new Section on Pharmacology organized in 1959. An attempt at making this the first joint program between the IUPS and the IUB, while viewed favorably by a majority of the officials of both unions, could not be consummated because of limitations of time. The preponderant flavor of the meeting, however, is to be biochemical.

The theme of this meeting—The Mode of Action of Drugs—actually is one which has stimulated outstanding efforts of distinguished workers, not only in pharmacology, but in physiology, including general physiology, biophysics, endocrinology, and radiobiology; biochemistry, including physical chemistry, histochemistry, enzymology, and nutrition; and pathology, in-

cluding microbiology, immunology, oncology, and toxicology. Dealing with an explanation of the effects of chemical substances on living systems, it represents as close an approach as one can find to a common interest among all branches of experimental biology. Pharmacology still has the same opportunity and challenge it has had since Buchheim and Schmiedeberg, of serving as a final common pathway for all the medical sciences. It can live up to these only by diversification, not by specialization in one type of approach or methodology.

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# ENERGY METABOLISM<sup>1</sup>

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## ENERGY METABOLISM AT THE MOLECULAR LEVEL

The extent to which over-all metabolic rate can now be accounted for by quantitative studies of the separate steps in intermediary metabolism is brought out in an inspiring review by Krebs & Kornberg (1). The review is largely concerned with the pathways and thermodynamics of intermediary metabolism (including photosynthesis), but the authors discuss several areas of interest in the present context.

(a) They write:

Surveying the pathway of degradation of foodstuffs as a whole, one cannot but be impressed by the relative simplicity of the arrangement inasmuch as the total number of steps required to release the available energy from a multitude of different substrates is unexpectedly small.

(b) The energy-yielding steps (as opposed to stages in degradation) amount to only six types.

(c) A diagram indicates the free energy available from each stage of glycolysis and shows that major quantities of energy become free only when ATP is hydrolyzed to ADP and P. Krebs & Kornberg write:

Hence the 'build-up' of energy-rich phosphate bonds must not be taken to mean that energy is in any way accumulated. It means that chemical changes are so organized that a major amount of energy can be released in one reaction, a gradual loss in each step being avoided.

This last statement is especially interesting in differing from the common view of energy-yielding reactions in living matter.

(d) A discussion of "control of energy-supplying processes" is concerned with the "primitive" pacemaker mechanisms upon which the nervous and endocrine control is superimposed in higher animals. The authors state:

Knowledge of intermediary metabolism and of enzyme systems has sufficiently advanced in recent years to prepare the ground for a study of the enzymic mechanisms which operate in the rate control of metabolic processes, and to give tentative answers to the question of how cells adapt their rates of energy supply to changing needs.

Various possible pacemakers are discussed in some detail.

(e) The appendix, by Burton, comprises an invaluable list of "free-energy data of biological interest".

<sup>1</sup> The survey of literature pertaining to this review was concluded in Spring 1960.



## WORK AND METABOLISM

*Muscular Work.*—Hill (2) recently discussed the idea that a muscle is able not only to produce, but also to absorb, work and that this absorbed work can reverse chemical processes that normally lead to the transfer of chemical energy to work. Work applied to a muscle (sometimes somewhat mystifyingly called "negative work") can thus be transformed to chemical energy. This intriguing idea is the culmination of an interesting development.

About a century ago, Heidenhain [Fenn (3)] noted that the heat produced by a muscle twitch increased with increasing load on the contracting muscle, and Fick (4) stated "that it is the actual process of doing mechanical work (shortening under tension) which is responsible for the chief expenditure of chemical energy in the muscle." Twenty years later, Hill (5) improved the technique of measuring the relation between work and heat production in muscle. In contradiction to Fick's conclusion, Hill maintained that the chemical energy expended by a stimulated muscle (transformed to heat and elastic energy) depended on the condition of stimulation and not on the mechanical work performed after stimulation. The total production of work and heat was the same for isotonic and isometric twitches. Again, ten years later, Fenn (3), working in Hill's laboratory, confirmed Fick's rather than Hill's conclusion. Fenn summarized: "When a muscle lifts increasing weights to a constant height the increase in heat obtained is roughly proportional to the weight", and further, "When a muscle lifts the same weight through increasing heights the heat obtained is roughly proportional to the amount of shortening."

In a complete muscle twitch (including a return to the prestimulation state) the heat obtained includes the work performed because on completion of the cycle, that work is transformed to heat. The heat obtained thus indicates the transfer of chemical energy by katabolism. Fenn concluded as follows: "Whenever a muscle shortens upon stimulation and does work by lifting a weight, an extra amount of energy is mobilized, which does not appear in an isometric contraction." "Isometric contraction" is a well established but unfortunately self-contradictory term. In an isometric twitch the contraction of one part of the muscle is combined with an equal stretch of the other (elastic) part, so that the muscle maintains a constant length. But a contraction cannot be isometric.

In a later article Fenn (6) gives a very lucid analogy. He writes: "It is suggested that contraction (of a muscle) is analogous rather to the winding up of an anchor chain by a windlass than to the lifting of a weight by the energy of a stretched spring." The muscle adjusts the extent of katabolic processes to the load which in Fenn's words it "discovers" it must lift after the stimulus for contraction is over.

Recently Hill & Howarth (7) confirmed Fenn's observation of the extra energy mobilized in a contracting muscle above that in an isometric twitch. They discuss the possibility that stretching a stimulated muscle leads to a partial reversal of the process by which chemical energy is transformed to

work. Thus mechanical work applied to a muscle may be partly transformed to chemical energy (as mechanical work applied to a generator produces chemical energy in a battery).

This transfer of mechanical to chemical energy, however, is limited. Hill & Howarth noted that the total heat produced during the twitch of a stretched muscle was never smaller than the heat equivalent of the work done by the ergometer in stretching the muscle. They concluded, therefore (p. 183), that the chemical reversal on stretching a muscle "is concerned only with the chemical products of the actual contraction in which the stretch was applied."

They explain the decreased heat production of a stretched muscle by the "thermoelastic effect of a change in tension", noting that this expression is a misnomer because the effect does not involve elasticity. Increase in pressure on a fluid with a positive coefficient of thermal expansion produces heat; decrease in pressure by stretching absorbs heat.

Muscle physiologists use "liberating energy" as a synonym for katabolism. One might think of this liberation as the opening of chemical bonds, but difficulties would arise if one were to conclude that liberation makes free energy out of bound energy, in the thermodynamic sense. On the contrary, the heat production during katabolism leads to a decrease in free energy. The danger of confusion may not be serious, but Hill's use of the term "net energy" for the difference between heat production of the muscle and the "negative work" (that is the work done by the ergometer on the contractile part of the muscle) is rather unfortunate. Net energy is a term introduced by Armsby (8) and frequently used in animal energetics. It is the difference between the metabolizable energy supplied by a feed and its heat increment (also known as specific dynamic effect). Armsby's net energy decreases with increasing heat production of the animal. Hill's new net energy increases with increasing heat production of the muscle.

Hill (2) extends his consideration of the reversal of chemical processes to observations of a man and a woman on bicycles arranged in opposition, so that the man pedaled forward and the woman resisted the rotation of her pedals. The man became exhausted while the woman remained fresh, showing that even in physiological functions, foot-dragging reaction is cheaper than progressive action.

Hutt *et al.* (9) manipulated various joints in the human body so that the motion was passive. They noted that the ventilation rate (minute volume) was increased without an increase in the rate of oxygen consumption.

Huxley (10) discusses three hypotheses attempting to explain muscle contraction by correlating structural observations and the thermodynamic approach. He writes (p. 279), "So far there is nothing worthy of the name of evidence either for or against any of these three general possibilities."

*Energy expenditure during various activities.*—Booyens & McCance (11) reinvestigated individual variations in human expenditure of energy in basal metabolism and the effect of sitting and standing on the metabolic rate.

When lying, the men had an average metabolic rate of 37.6 kcal. per m.<sup>2</sup> of the DuBois surface per hr. This would be 900 kcal. per m.<sup>2</sup> per day and is in close agreement with the results of Harris & Benedict (12) (926 kcal. per m.<sup>2</sup> per day). The basal (lying) metabolic rate of the women investigated by Booyens & McCance, 35.6 kcal. per m.<sup>2</sup> per hr. or 853 kcal. per m.<sup>2</sup> per day, is in even closer agreement with the mean of the women tested by Harris & Benedict (848 kcal. per m.<sup>2</sup> per day).

Sitting instead of lying increased the metabolic rate of men from 37.6 to 42.0 kcal. per m.<sup>2</sup> per hr. and that of women from 35.6 to 41.6 kcal. per m.<sup>2</sup> per hr. Standing caused a further increase in the metabolic rate to 46.4 kcal. per m.<sup>2</sup> per hr. in men and to 46.6 kcal. per m.<sup>2</sup> per hr. in women.

Booyens & Keatinge (13) observed that women not only have a relatively lower basal metabolic rate than men but that they also spend less energy for walking than men do. They measured the rate of oxygen consumption of 10 men and 10 women walking 500 yards, first at a leisurely pace (5.52 km. per hr. for the men, 5.44 km. per hr. for the women) and then at a "catch a bus" speed (6.52 km. per hr. for the men, 6.48 km. per hr. for the women).

During the leisurely walk the men had a metabolic rate of 4.93 kcal. per hr. per kg. body weight; the women spent 4.49 kcal. per hr. per kg. body weight. The latter is 4.5 times as great as the estimated basal metabolic rate ( $65.8 \cdot W^{3/4}$  kcal. per day).<sup>2</sup> During the faster walk (6.5 km. per hr.) the men increased their metabolic rate to 5.86 kcal. per hr. per kg. and the women to 5.12 kcal. per hr. per kg. The men had not only a higher energy expenditure than women during the slow walk, but with increased speed, they increased their metabolic rate 19 per cent while the women increased their metabolic rate only 14 per cent.

According to Booyens & Keatinge, women can walk more economically than men (per kg. body weight) because they take shorter steps both absolutely and relatively. The ratio of length of stride to length of leg averaged 0.91 for the men and only 0.86 for the women. For the increase in speed of walking from 5.5 to 6.5 km. per hr. the men increased their relative stride from 0.91 to 1.00 (10% rise) and increased the frequency from 106.7 to 114.9 strides per min. (7.7% increase). The women, on the other hand, increased the relative length of stride from 0.86 to 0.91 (only a 6% increase), but they increased the frequency from 126.4 to 141.1 strides per min. (12% increase).

From an article by Benedict & Murschhausen (14) and the book by Steindler (15), Booyens & Keatinge conclude that

The longer the stride the nearer the pelvis falls to the walking surface. Thus the work performed in the vertical plane was increased when the stride was lengthened. It seems likely therefore that the lower expenditure of energy of the women was due to the smaller amount of work they performed in lifting their bodies vertically.

At first sight this simple geometrical explanation of the more economical

<sup>2</sup> According to Harris & Benedict's measurements women produced daily 23.9 kcal. per kg. (12); this would be 1.0 kcal. per kg. per hr. for women. The corresponding figure for men is 25.5 kcal. per day per kg. or 1.06 kcal. per kg. per hr.

walking of women seems very convincing, but it becomes less so when one considers that the shorter steps are correlated with a higher frequency. Women do not have to lift the center of gravity as high as men but they have to lift it 23 per cent more often.

Nevertheless if we analyze this work of walking as a greatly simplified problem of elementary mechanics (assuming stiff legs), we can calculate that the decrease in amplitude (a sine function of the angle between the horizontal and the leg at full stride) more than compensates for the necessary increase in frequency (which is inversely proportional to the stride length). For these highly simplified conditions we can calculate the relations given in Table I.

TABLE I

Relative stride length	1.00	0.95	0.90	0.85	0.80
Relative amplitude of vertical oscillation	100	89.6	79.8	70.9	62.7
Frequency $\left(\frac{100}{\text{relat. stride}}\right)$	100	105	111	118	125
Work (frequency $\times$ amplitude $\times 10^{-2}$ )	100	94.1	88.6	83.7	78.4

Thus, even if the frequency increase is properly taken into account, shorter and more frequent steps appear energetically more economical.

The most economical walking is that with the least vertical oscillation, but this can also be varied by the degree of knee bending or of horizontal oscillation.

Durnin & Namyslovski (16) found no difference between the energetic efficiency for walking of women and men.

*Work and obesity.*—McKee & Bolinger (17) measured the oxygen consumption of 44 persons of both sexes between the ages of 18 and 66. Nineteen of the subjects exceeded the relative weight of 110 per cent of normal (according to Metropolitan Life Insurance standards) and were considered obese.

The basal metabolic rate was measured during 5 min. This measurement was followed by a 15-min. work period, during the last 5 min. of which the oxygen consumption was measured again. The difference between the rate while working and while resting is the energy expended for work. (In Fig. 2 it is stated erroneously that the area in the plot of metabolic rate vs. surface area represents this expenditure.) The work consisted of lifting a 9-lb. weight 1 ft. high every 2 sec. From the authors' data one may calculate that the partial efficiency for mechanical work ( $\Delta \text{work} / \Delta \text{katabolism}$ ) of the nonobese males in these trials amounted to 1.3 per cent.<sup>3</sup>

The authors state that the basal metabolic rate is significantly greater

<sup>3</sup> 30 ft. lb. per min. = 150 ft. in 5 min. = 48.6 cal. of work in 5 min. Increase in katabolism of normal males in 5 min. = 3.7 kcal. (McKee and Bolinger, Table I); thus efficiency  $(48.6)/(3.7 \times 10^3) = 1.3 \times 10^{-2} = 1.3$  per cent.

for obese persons of both sexes than for the nonobese subjects. Checking the table from which the authors draw this conclusion, we find that for males the difference is insignificant. Since the body weights are not stated, one cannot evaluate the difference among the females because the obese females may have been heavier (absolutely) on the average than the normal females. The table with the correlation coefficients of surface area to metabolic rate is no substitute for the knowledge of the weights. The figures also would have been more useful with the actual results as dots (possibly with the regression lines added), giving more information without increased printing space. This type of incomplete reporting has been criticized by Kleiber (18) in an earlier *Annual Review of Physiology*.

McKee & Bolinger (17, p. 199) state, "In general increasing surface area correlates with increasing obesity." This is contrary to what one generally expects, namely, that obese people are more stocky and consequently have lower Meeh constants [ $K = (\text{surface})/(\text{weight}^{2/3})$ ]. This is the rational basis for comparing relative surface areas. The authors obviously deal with absolute surface areas. Possibly the obese subjects were on the average heavier than the normal subjects, and even with lower relative surface areas (Meeh constants) they had greater absolute surface areas, just as a 1-kg. steel ball may have an absolutely greater surface area than a needle, or a starved horse a higher (absolute) fat content than an obese mouse.

The authors arrive at two apparently contradictory conclusions: (a) that with increased surface area (which they claim correlates with increasing obesity), the females become less efficient and the males more efficient for work; (b) that there is no deviation from normal in the work efficiency of obese persons.

The efficiency of energy utilization for horizontal walking, according to a recent investigation of Ralston (19), has a maximum at a given speed. This is the speed which persons choose if asked to walk at a pace which seems natural to them. Ralston expressed the relation between the power (energy per unit time) necessary for horizontal walking per unit weight as a parabolic function of the velocity as follows:

$$E_w = 29 + 0.0053v^2$$

in which  $E_w$  is expressed as cal. per min. per kg. body weight, and  $v$  is velocity in m. per min.

As would be expected, the standing metabolic rate for men, 29 cal. per kg., is considerably higher than the basal metabolic rate of 17.7 cal. per kg. per min., as measured by Harris & Benedict (12).

According to the equation above, the energy expenditure per kg. body weight of a standing man is independent of his body weight, but presumably the standing metabolic rate of man depends on body size. For a population with only small differences in body weight (as among adult men), the size effect on the metabolic rate per unit weight may be neglected. If the effect of body size on the standing metabolic rate is the same as that on the basal metabolic rate, and if this effect follows the rule derived by interspecific com-

parison of animals from rat to horse, then the metabolic rate per unit weight is inversely proportional to the fourth root of body weight. A doubling of the body weight, therefore, would lead to a decrease of only 16 per cent of the metabolic rate per unit weight.<sup>4</sup>

#### TEMPERATURE REGULATION

*Effect of temperature on lower animals.*—Roberts (20) noted that, with increasing environmental temperatures, the increase of metabolic rate of crabs follows Van't Hoff's law between 16° and 23.5°C. The Van't Hoff quotient ( $Q_{10}$ ) increases directly with body weight. Dawson & Bartholomew (21) made similar observations on the lizard *Dipsosaurus dorsalis*, in which the rate of oxygen consumption also increases with environmental temperature according to Van't Hoff's law; at environmental temperatures between 19° and 45°C., the Van't Hoff quotient was 2.5. The frequencies of breathing and of heart beat were similarly affected by changes in environmental temperature.

A very interesting type of physical temperature regulation has been observed by Bartholomew (22) in *Setonix brachymus*, a marsupial of South-western Australia. Instead of sweating, as some more advanced creatures in the line of evolution do, these marsupials "salivate heavily and lick their front and hind feet, legs, tails and sometimes bellies until these parts are dripping wet." The consequent evaporation prevents the overheating of the animal. Apparently the licking is stimulated through peripheral receptors and not from an elevation of the internal temperature. These marsupials also have a metabolic temperature regulation against cooling, and as in higher animals, the increase in metabolic rate is accompanied by shivering.

Newborn mice are not yet homeotherms but neither do they act entirely as poikilotherms. Fitzgerald (23) noted that their rate of oxygen consumption as a function of the environmental temperature does not fit the Arrhenius equation, whereas their respiratory frequency does.

*Temperature regulation in birds.*—Dawson (24) has studied the temperature regulation of the cardinal, a passerine bird with a body weight ranging from 38 to 48 gm. The rate of oxygen consumption after 3 hr. of fasting amounted to 2.6 ml.  $O_2$  per hr. per gm. body weight. This corresponds to a metabolic rate of 0.53 kcal. per hr., which is relatively high, since according to the rule of body size and metabolic rate (3 kcal. per hr. per kg.<sup>3/4</sup>), an animal weighing 43 gm. would have a basal metabolic rate of only 0.28 kcal. per hr. Between environmental temperatures of 24°C. to 34°C., the metabolic rate was independent of changes in temperature; above this limit the metabolic rate increased about 8 per cent of the level at thermal neutrality for every degree C. rise of environmental temperature above 34°. The cardinal has a very well-developed metabolic (chemical) temperature regulation; below an environmental temperature of 24°C., a decrease of 1°C. produces an increase in metabolic rate of 5.6 per cent of the rate at thermal neutrality.

<sup>4</sup> That is:  $(1/2)^{1/4} = 0.84$ .

The rate at thermal neutrality is not significantly affected by the season, and Dawson concludes that the behavior of the cardinal in this respect is similar to that of the other birds and mammals in Alaska. For physical temperature regulation the cardinal relies largely on increased water evaporation by panting. In a hot environment, however, this method of heat dissipation may become inadequate and then the body temperature rises, but the cardinal seems able to tolerate several degrees increase in body temperature.

Irving & Krog (25) observed some uniformity in the body temperatures of 29 Alaskan species of birds. They noted, however, considerable fluctuations of body temperature during the day. Alaskan birds cool as much as 3° by night, and with activity they can raise their temperature as much as 3°C. This is almost as great a range of body temperature as that observed by the Schmidt-Nielsens (26) in the camel.

The embryo in the egg and the newly hatched nestlings do not have temperature regulatory mechanisms of their own but they are homeothermic in that they require a constant temperature for survival. The parents keep the temperature of the eggs and the young within even narrower limits than those within which they regulate their own body temperature when away from the nest. This is true in Alaska as well as in warmer regions. Removed from the regulatory activity of the parents, however, newly hatched birds are poikilotherms. This was shown clearly by Dawson & Evans (27) who studied the relation of growth and development to temperature regulation in sparrows. They report that in one- and two-day-old birds, oxygen consumption varied directly with environmental temperature from less than 0.5 ml. O<sub>2</sub> per gm. per hr. at 17°C. to 3.5 ml. O<sub>2</sub> per gm. per hr. at 35 to 40°. On the fourth day after hatching, the birds began to defy Van't Hoff's law with respect to environmental temperature. They maintained a quasicontant metabolic rate over a range of 25° to 37°C. By the fifth day the nestlings began metabolic temperature regulation; they increased their metabolic rate with decreasing environmental temperature below 35°. This development preceded the growth of feathers, which are important for physical temperature regulation. In ontogenesis, therefore, the chemical temperature regulation begins to function before the physical temperature regulation.

Irving & Krog (25) measured the temperature in nests with young birds in the arctic. They conclude their investigation of seven species with the following statement: "The stable, warm temperature for early avian development is common to all climates and it is maintained through an equally common prescription for temperature regulation by parental behavior."

This temperature regulation by parental behavior is not absolutely safe, even in *Homo sapiens civilis*. Mann & Elliot (28) write that neonatal cold injury (to human babies) is a not uncommon disorder. "Prevention which is of paramount importance because of the high mortality, can be achieved only by safeguarding against a fall in room temperature. This requires watchfulness by professional personnel who should warn parents of the risk."

*Temperature regulation in mammals.*—To help out in their temperature regulation, rats can learn to operate an air conditioner. This was shown by



Hamilton (29) who trained rats to depress a response bar when they desired more heat. With this device it was observed that rats exposed to low ambient temperatures called for more heat from their air conditioner before feeding than after feeding.

Swanson (30) investigated the effect of thyroxin and epinephrine on the metabolic rate of albino rats at different environmental temperatures. She noted that increasing doses of thyroxin decreased the rat's metabolic response to cooling below the critical temperature (for the rat this is near 30°C.).

The Schmidt-Nielsens (26) have published some additional data confirming their earlier observation of the great variability of a camel's body temperature and the advantage of this variability for water conservation. Temperature regulation by evaporative cooling in the camel operates almost exclusively through perspiration rather than pulmonary ventilation. In a hot environment a shorn camel loses more water by evaporation than a camel protected by its fur, which acts as a barrier against heat gain from the environment. Under the same heat load, a donkey evaporates more water per day per unit of body weight than does the camel. A part of the difference may be a size effect because the donkey is smaller and therefore has a higher metabolic rate per unit body weight. For a complete picture of the situation, the rate of evaporation should also have been expressed per unit of the metabolic body size, not to replace, but to supplement the information given in the Schmidt-Nielsens' figure.

Iampetro *et al.* (31) studied the effect of food, climate, and exercise on the daily fluctuations of the rectal temperature in man. They observed that the major portion of the changes in rectal temperature during the day is closely correlated to food intake.

Hammel *et al.* (32) have adapted an ingenious technique of Lilly (33) by which needle thermodes carrying warm or cold water can be painlessly inserted via chronically implanted metal guides into the hypothalamus of an unanesthetized dog. Among other observations, they noted that rapid alternate heating and cooling evoked panting and shivering simultaneously. Scholander & Krog (34) have published a study of a heat exchange mechanism in the sloth. In this and a few other species, the limbs and tail contain arteriovenous bundles (retes), in which there is countercurrent heat exchange. The arterial blood supplying the extremities thus first warms the returning venous blood, thereby conserving body heat at the expense of cooler limbs.

*Metabolic temperature regulation without shivering.*—Rubner distinguished two types of animal temperature regulation. One type, physical temperature regulation, influences the rate of heat loss, and the other type, chemical temperature regulation, influences the rate of heat production. In man and dog the increased heat production due to chemical temperature regulation seems always related to muscular contractions, usually noticeable as shivering. Krogh (35) reported that a curarized dog lost the ability for chemical temperature regulation and became poikilothermic with a metabolic rate



following Van't Hoff's law. Johansen (36) made experiments on himself and learned to suppress shivering during cooling. His metabolic rate did not rise in the cold until the shivering reflex overcame his will power. Some physiologists concluded from these results that the increase in heat production as a response to cooling operated only through shivering, and some concluded further that in this case there was no "true" chemical temperature regulation. This latter conclusion obviously follows a misunderstanding of Rubner's terms; any increase in the rate of animal heat production means an increase in the rate of katabolic, that is chemical, processes whether or not the heat production is correlated with muscular activity.

It is known now that chemical temperature regulation can occur without shivering, especially in the rat, and several authors designate that part of the metabolic increase as "chemical" [Hart (37)]. This terminology implies that the increase in metabolic rate with shivering is not chemical. It is obviously preferable to avoid such an unsound implication and speak of metabolic temperature regulation with or without shivering, rather than of chemical and nonchemical metabolic temperature regulation.

Weiss (38) measured the oxygen consumption of rat tissue slices. He noted that the *in vitro* metabolism of tissues from cold-exposed rats is higher than that of rats kept at normal temperature. This observation does not necessarily mean that the metabolic rate of homeotherms in general or the thermostatic rise in their metabolic rate is controlled by the cells per se. It is also consistent with the conclusion of Kleiber & Cole (39) that the respiration of tissues *in vitro* is still affected by the previous condition of the animal and that the conditions in the cells produced by the action of the central regulator for temperature are still prevailing *in vitro*. The increase in metabolic rate may well be caused by increased concentrations of respiratory enzymes in the cells. The observations of Weiss (38) show that increases in muscular heat production following cold exposure are possible without muscle contraction. We assume that the tissue slices *in vitro* do not shiver. Since the metabolic rate of surviving tissues reflects the *in vivo* metabolic rate [Field *et al.* (40); Martin & Fuhrman (41)], a general conclusion from the observation of Weiss is that shivering is not a necessary condition for the thermostatic rise of metabolic rate even in those animals in which metabolic temperature regulation is always accompanied by shivering. Shivering in this case may be only one facet of chemical temperature regulation rather than the sole means of it. The largest *in vitro* increase in metabolic rate of tissues from cold-exposed rats was noted in liver slices. This observation supports the suggestion of Hsieh & Carlson (42) that a considerable part of nonshivering calorogenesis in cold-exposed animals may occur in the liver. Depocas (43), on the other hand, reports from experiments with functionally eviscerated rats that the presence of viscera is not necessary for nonshivering calorogenesis in the cold-acclimated rat.

In an earlier paper, Depocas *et al.* (44) found that among rats exposed for 2.5 hr. to  $-5^{\circ}\text{C}$ ., those that were cold acclimated showed a lower relative

oxidation of injected glucose and a greater relative glycogen synthesis from this glucose than the rats that were not cold adapted. This is in line with the several observations that the cold-adapted animal develops a more efficient temperature regulation.

Hsieh & Carlson (42) noted that cold-exposed rats katabolized thyroxine twice as fast as did rats kept at a normal temperature. The increase in metabolic rate continued even when the store of thyroxine was exhausted, and these authors conclude that thyroxine is not the key substance in metabolic temperature regulation. They therefore investigated the role of epinephrine and norepinephrine in the chemical regulation of heat production [Hsieh & Carlson (45)]. Shivering was precluded by administration of curare or by transection of the spinal cord. Some of the rats were cold adapted by keeping them at 5°C. for 17 to 30 days; others were kept at 30°C. The oxygen consumption of all the rats (both normal and cold-adapted) was measured at 30°C. The cold-adapted rats had an oxygen consumption of 950 cc. per hr. per kg.<sup>3/4</sup> (The latter amounts to 3 kcal. per hr. per kg.<sup>3/4</sup>, which is exactly the interspecific mean for basal metabolic rate [Kleiber (46)]). Thyroidectomized cold-adapted rats consumed 490 cc. per hr. per kg.<sup>3/4</sup> and thyroidectomized warm-adapted rats only 370. Injection of epinephrine increased the metabolic rate of cold-adapted rats by a maximum of 80 per cent and that of the warm-adapted rats by 48 per cent. The corresponding effects of epinephrine injection after thyroidectomy were 35 and 32 per cent, respectively. In normal rats epinephrine induces more calorogenesis than norepinephrine, and vice versa in cold-adapted rats. The metabolic effect of norepinephrine more nearly resembles that of cold exposure than does the effect of epinephrine. Hsieh & Carlson suggest, therefore, that norepinephrine may be the mediator in (nonshivering) metabolic temperature regulation. These authors further noted that the curarization of their animals did not significantly affect either the metabolic rates or the increase in metabolic rate with decreasing environmental temperature. This is in contrast with Krogh's (35) earlier findings with dogs.

Hart (37), describing the rat's increase in rate of heat production resulting from metabolic temperature regulation, states: "This striking demonstration of acquired heat production without muscle contractions during acclimation to cold has not been found in any other species." It still seems unlikely that rats are the only species which can operate chemical temperature regulation without shivering.

Werner *et al.* (47) apparently believe that they have found a nonshivering metabolic temperature regulation in dogs. They anesthetized dogs with sodium thiopental, cannulated the trachea and blood vessels, and administered 10 per cent of a lethal dose of curare. They then cooled the dogs in an ice bath until the rectal temperature dropped to 29°C. (artificial respiration was applied). When this cooling was accomplished, the metabolic rate was 52 per cent of the normal metabolic rate of control dogs. The investigators then removed the cooled dogs from the ice bath, dried them, and

wrapped them in blankets to minimize heat loss. They then noted a rise in temperature. According to their introductory remarks, the authors seem to conclude that their experiment indicates metabolic temperature regulation without muscular activity. This conclusion is not justified. The extent of chemical (or metabolic) temperature regulation is measured by the difference in metabolic rate of homeotherm at a low environmental temperature and its metabolic rate above the critical environmental temperature.

What the experiments of Werner *et al.* (47) do, in fact, show is that with artificial respiration a curarized dog can continue to consume oxygen for some time. This oxygen consumption is accompanied by heat production, and this heat production, even though below the normal level, can increase the temperature of the body if heat loss is minimized with a woolen blanket. In this case, the experimenters did not actually observe the dog's temperature regulation, but by wrapping the dog in a blanket, they substituted for it by decreasing the rate of heat loss. If the dog had done it, it would be classified as physical rather than chemical temperature regulation.

From a comparison of the electromyogram and the curve for oxygen consumption, Froese (48) concluded that exposure to cold in man can start an increase in metabolic rate before a tremor is recorded. This is consistent with the idea that shivering in man may contribute only a part of, rather than a necessary condition for, the thermostatic rise in metabolic rate. This author also observed a significant decrease in the extent of response in the metabolic temperature regulation when the human subject was breathing oxygen instead of air. (At 25° the metabolic rate when breathing air or O<sub>2</sub> was the same.)

*Man's critical temperature.*—Swift (49) concluded his report of metabolic rates of men at various environmental temperature with the statement: "Shivering begins when the skin attains a temperature of approximately 19°C."

If we assume that metabolic temperature regulation in man is accompanied by shivering, we can conclude from Swift's results that an increase in metabolic temperature regulation starts when the skin temperature drops to 19°C. Assuming that the air temperature is not higher than the skin temperature, we would then conclude that the critical temperature for man is 19°C.

The more recent results of Erikson *et al.* (50), however, led them to the conclusion that a nude man's critical temperature is approximately 26°C. Scholander *et al.* (51), working with Lapps, came to the same conclusion. They found that Lapps without clothes have a critical temperature of 27°C., but in their winter clothing their critical temperature is -10°C. On the other hand, Kandror (52) found that 12 healthy subjects in the Russian arctic showed increased basal metabolic rates in winter.

The apparent discrepancy between the results of Swift and those of the Norwegian physiologists may mean that decreasing environmental temperature first produces an increase in metabolic rate before shivering starts and that, therefore, in the neighborhood of the critical temperature, man has a thermostatically induced increase in metabolic rate without shivering.

*Heat loss in man.*—Froese & Burton (53) showed that the head is an important source of heat loss. At an environmental temperature of  $-4^{\circ}\text{C}$ . the heat loss via the head may amount to as much as one-half of the basal metabolic rate. This confirms the observations of Bohnenkamp (54) that the forehead is the most radiant part of a human body.

*Adaptation to cold.*—Adaptation means "to make fit" [Carlson (55)], but what is meant by being fit? Scholander (56) gives a suitable criterion as follows: "It can fairly be stated that men and animals are only fully adapted to their environment provided they can rest and sleep in it."

The American Physiological Society held a symposium entitled "The Metabolic Aspects of Adaptation of Warm Blooded Animals to Cold Environment" in 1958 (Chairman, L. D. Carlson). The papers presented and the discussion were published in the *Federation Proceedings* (56a).

Hart (57) reported that rats kept in a cold environment first double their metabolic rate by shivering; then this shivering gradually decreases and, after 30 days of cold exposure, disappears. During this period, nonshivering calorogenesis increases so that finally all extra heat necessary for maintaining body temperature is provided by increased katabolism without shivering. This nonshivering increase in metabolic rate may be accompanied by changes in intermediary metabolism. Smith & Fairhurst (58) measured the *in vitro* oxidation and phosphorus esterification rates in liver slices from cold-adapted rats. They summarized their results as follows:

It is suggested that the increased heat production of the cold-adapted mammal is a direct function of an altered utilization of the energy derived from oxidative metabolism wherein a physiological uncoupling occurs resulting in a net decrease in phosphorus esterification as measured *in vitro*.

Other indications of changes in the patterns of metabolism during adaptation to cold include the observation that cold-adapted rats have decreased hepatic lipogenesis and that they incorporate more  $\text{C}^{14}$  from labeled glucose into glycogen than comparable controls, both exposed to a temperature of  $-5^{\circ}\text{C}$ . [Masoro *et al.* (59)].

Hannon & Vaughan (60) have systematically assayed the effect of cold exposure on the glycolytic enzymes of rat liver and muscle. The elevated levels of some enzymes lead the authors to conclude that cold-exposed rats have enzymic capacities for increased utilization of free glucose, increased glycogenolysis, and increased gluconeogenesis. They note decreased hexose monophosphate shunt activity.

Potter (61) presented a scheme to show how adaptation in general may change the rates of biochemical processes. He suggests a feedback of metabolites affecting (a) deoxyribonucleic acids (DNA), representing genes, leading to evolutionary adaptation; (b) ribonucleic acids (RNA) which comprise enzyme-forming systems, for slow physiological adaptations; and finally (c) enzyme systems themselves and substrates of the enzymatic processes for fast physiological adaptations.

Ingle (62), discussing the endocrine systems involved in adaptation to a

cold environment, emphasized the interaction of these systems and commented, with a pertinence not limited to cold adaptation, "When a function fails to disappear following removal of an organ it is unsafe to assume that the organ had nothing to do with the function", further: "When a function disappears upon removal of an organ it is unsafe to conclude that it was a function of this organ."

Ingle sums up the present state of our knowledge of the role of endocrine glands in adaptation to cold as follows:

It is reasonably clear that heat production and adaptation are not controlled completely by one gland. The presence or absence of hormones of the pituitary, thyroid and adrenal glands can affect heat production and the success of adaptation. The role of these hormones in adaptation may be permissive, or it remains possible that a very active role in the regulation of heat production can be attributed to any one or several hormones.

Desmarais (63) has written an extensive review of his own work and that of others which indicates that an animal exposed to cold is benefited by ascorbic acid. Ascorbic acid administration can prevent the hypertrophy of the adrenal cortex which otherwise results from exposure to cold. Desmarais noted a parallelism between the effect of ascorbic acid and DOCA (deoxycorticosterone acetate). He assigns to ascorbic acid a role in the first line of defense against cold, a defense which mainly involves the activity of the thyroid gland, whereas the hyperactivity of the adrenal cortex represents a second line of defense.

Héroux *et al.* (64) compared the metabolic rate and pelt growth response of rats exposed to cold outdoors and in the laboratory. The pelt's insulation increased in rats kept outside but remained unchanged in rats kept at 6°C. in the laboratory. The resting metabolic rate (measured at 30°C.) increased in the latter animals but not in those kept outside.

*Man's adaptation to cold.*—On the basis of his definition of adaptation to cold (see above), Scholander (56) distinguishes three different methods by which man can adapt. For other animals, a fourth can be added:

- (a) Technological adjustment of the microclimate.
- (b) Metabolic and psychological adjustment—learning to sleep while shivering.
- (c) "Insulative cooling", that is an extension of physical temperature regulation.
- (d) An increase of the specific insulation by growing thicker fur, of which the classical example is Hoesslin's dog [Hoesslin (65)]. This type of slow adaptation to a cold environment has been lost by man. It is like insulative cooling, an extension of physical temperature regulation. Hart (37) termed it insulative adaptation.
- (a) Adjustment of microclimate. In comparison with many other animals, man has a rather limited physiological adaptability. To exist under adverse climatic conditions he has to rely mainly on his intelligence for technological

adaptation. Scholander *et al.* (51) have made measurements showing that Eskimos and Lapps construct their living quarters and make their clothing so that even during the Arctic winter their microclimate differs little from that of ordinary inhabitants of temperate regions.

(b) Metabolic adaptation. Man, especially during youth, has the drive to challenge nature in a more primitive way than by technology. Instead of adjusting his microclimate he can learn to sleep while shivering. This was demonstrated during an autumn expedition above the tree line of the Norwegian mountains [Scholander *et al.* (66)], in which men slept in a single-blanket sleeping bag at about freezing point. Scholander summarized the result as follows:

In the beginning the men suffered from cold, especially the feet, shivering and thrashing about all night, but after about a week they were able to sleep through the night and they stayed warm from head to foot. Throughout the entire exposure period they compensated for the cold by raising their heat production, of which shivering was a conspicuous component and which soon became compatible with sleep and rest.

A paper by Kreider & Buskirk (67) is of special interest in this connection. They measured rectal temperature, skin temperature, toe temperature, and metabolic rate of men sleeping in the cold. Rectal temperature and toe temperature, as well as rate of oxygen consumption, were significantly increased when the men ate a supplemental meal of 600 to 1200 kcal. (in addition to the regular three daily meals) ten minutes before retiring. The skin temperature was not affected by the extra snack, but the number of periods of wakefulness was decreased and the men felt better.

(c) Insulative cooling. Metabolic temperature regulation costs a lot of energy, especially when it is accompanied by shivering [Erickson *et al.* (50)]. Shivering decreases the resistance to heat flow from the interior to the body surface and increases the heat loss by convection from the body surface to the surrounding air, as DuBois (68) showed with patients during malarial chills.

In some human societies which are blessed with neither agricultural surpluses nor a high degree of technological skill, and whose members have body coverings like Adam's and Eve's before they sinned, natural selection has developed a more economical method of cold adaptation. Scholander *et al.* (69) measured body temperature, skin temperature, and respiratory exchange in Australian aborigines resting naked in sleeping bags<sup>5</sup> at air temperatures ranging from 0 to 5 C. Unacclimated whites spent a sleepless night shivering and thrashing around, increasing their metabolic rate yet getting cold feet. Acclimated white men had an even higher metabolic rate; they also shivered, but they slept and had warm feet. The Australian aborigines had a metabolic rate slightly below normal; their feet were cold but

<sup>5</sup> The sleeping bags were necessary for the experiment; the Australians ordinarily sleep naked without a bag.

they slept soundly and did not shiver. Scholander calls this economic practice "insulative cooling".

The Bushman in the Kalahari desert of Africa makes more use of technology to maintain a suitable microclimate than the Australian aborigine does, but still does not shiver under conditions causing shivering in white men. Wyndham & Morrison (70), who studied the temperature regulation of the bushmen, suggest at least the possibility that these people have some form of climatic adaptation which is not found in Caucasians.

Under special circumstances, something of this most primitive, but also most economical, method of dealing with a cold environment might possibly be reactivated again even in Caucasians (at least in Norwegians!).

In Nansen's inspiring book (71) on the Norwegian Polar Expedition of 1893-1896, one reads, Vol. 2, p. 70: "The weather appears quite mild when one now sits . . . and sews at a temperature of  $-28^{\circ}\text{C}$ . in comparison with the earlier temperature of  $-40^{\circ}\text{C}$ . when it was no pleasure to handle the needle," or on p. 81, "It was heavenly in this mild weather ( $-11.3^{\circ}\text{C}$ ).". Later, p. 170, Nansen writes that a plug of the tent on the arctic ice had come loose and Johansen's bare foot stuck out in the open but he did not notice it and slept soundly. One wonders if during their trek across the ice, Nansen and Johansen had not also acquired to some degree the ability to stand some insulative cooling.

Wood *et al.* (72) indeed observed peripheral venous constriction after three days of exposure of five men to rather mild cold ( $60^{\circ}\text{F}$ .).

*Hibernation.*—To meet the heat requirement in a cold environment for any extended time the animal needs food, and if this source of energy is lacking the animal starves to death. The lower the environmental temperature, the shorter is the survival time. Small mammals, like the pocket mouse *Perognathus longimembris* may live in the cold as homeotherms as long as they have plenty of food. If the food becomes scarce they give up, but instead of dying they hibernate, or even aestivate [Bartholomew & Cade (73)].

Even during "deep hibernation", hibernants differ from poikilotherms in that their metabolic rate does not follow Van't Hoff's law. The metabolic rate of most hibernators reaches a minimum at  $5^{\circ}\text{C}$ . If the environmental temperature sinks below this level, the hibernator increases its metabolic rate while either continuing to hibernate or while waking up [Lyman (74)].

A welcome clarification of the term "hibernation" was supplied by Hock (75) in a discussion of metabolism and cold adaptation. He considers a "spectrum" of various degrees of hibernation. The body temperature of the bat drops  $40^{\circ}$ , that of the ground squirrel  $37^{\circ}$ , that of the prairie dog  $17.5^{\circ}$ , and that of the bear  $7^{\circ}$ . This reaction readmits the bear to the family of hibernators from which he had been kicked out. Hock also corrects the idea that hibernation is a constant condition. He shows that hibernation is a series of sleeps interrupted by short periods of activity. Periodic arousals are expensive of energy but presumably important to maintain conditions suitable for subsequent arousal; the level of certain metabolites in the body might



well be critical, for example. It is perhaps analogous to the periodic charging of the battery in a stored car so that it can be readily started in the future.

Chao *et al.* (76) noted that during hibernation a hedgehog loses 1.37 gm. of weight per kg. body weight daily. If we express the daily weight loss per unit of the metabolic body size ( $\text{kg.}^{3/4}$ ), we can calculate from Chao's results that a fasting man losing 0.6 kg. of weight per day has about 20 times the relative weight loss of the hibernating hedgehog.

Kayser (77) has reviewed over 400 articles on hibernation and concludes that the adrenal cortex is a key organ. Removal of this gland prevents hibernation; the adrenalectomized animal does not hibernate and dies of cold as nonhibernants do. Kayser suggests that the "hibernating gland" (which may be the adrenal cortex) produces a hormone "*hypnogène*" and that this hormone predisposes an animal whose fuel supply is not exhausted to let itself be cooled rather than fight cooling by increasing its metabolic rate as normal homeotherms do.

In the light of Ingle's (62) views, mentioned above, the search for any one hibernating gland or any one hormone may be in vain. Hibernators have possible the same hormones as nonhibernants, and the difference between hibernating and nonhibernating homeotherms might lie in different interactions of those hormones rather than in the presence or absence of one of them. This, however, should not discourage the search for a *hypnogène*, because it might exist.

#### REGULATION OF METABOLISM

*Body size and metabolic rate.*—Roberts (78) has confirmed the earlier observations of Weymouth *et al.* (79) and of von Bertalanffy (80) that the metabolic rate of crustaceans shows a relation to body size similar to that of the metabolic rate of mammals.

Fuhrman & Fuhrman (81) observed that the rate of oxygen consumption of rat skin *in vitro* depends on the body size of the rat, much like the *in vitro* rate of liver slices.

The influence of body size on metabolic rate still challenges physiologists to find other physiological correlates in this relationship. Schmidt-Nielsen & Larimer (82) report a small negative linear regression between the log of the half-saturation oxygen pressure in blood and the log of body weight. Furthermore, Larimer & Schmidt-Nielsen (83) found that the level of carbonic-anhydrase in the mammalian red blood cell is about 10 times as great in mice as in horses. In contrast to their findings however, Ganyushkina (84) reported that cows with relatively low metabolic rates had a higher level of carbonic anhydrase activity.

A recent comparative study of four means of expressing the metabolic rate of rats [Chiu & Hsieh (85)] contains a number of misconceptions and wrong conclusions. For example, the statement: "There is a gradual fall in metabolic rate of rats with increasing age from 7 to 19 weeks. This is due to increasing weight of the animal" or, "Rats kept in a cold environment of



6°C. have a higher metabolic rate than controls kept at 28°C. This is due to the lower body weight of the former group."

*Age and metabolic rate.*—In general, animals of different ages have different body sizes. Therefore the metabolic effect of age has a proper meaning only if expressed for a given unit of size. In contradiction to Chiu & Hsieh (85), we maintain that there is no single "correct" way of expressing metabolic rate with respect to body size. The various units of body size in which metabolic rates can be expressed are more or less suitable for what one wants to know—rate per rat for planning the heating equipment for a rat colony; rate per unit surface area for estimating insulating power of animal covering; rate per unit weight for getting an idea of relative rates of enzymatic processes; rate per unit of metabolic body size ( $\text{weight}^{3/4}$ ) for expressing metabolic levels in comparative physiology.

Kleiber *et al.* (86) have therefore expressed the metabolic rate of rats from birth to 1000 days of age, together with the body weight in four different units; per rat, per kg. body weight, per unit of estimated body surface ( $W^{2/3}$ ), and per unit of metabolic body size ( $W^{3/4}$ ). Since each of the 42 figures in each of the four columns in this report is a mean of original data, the results of the four columns cannot strictly be calculated one from another. Therefore, the presentation of metabolic data in this way, even though needing somewhat more space, is more useful than presenting only one figure and certainly more useful than expressing the results only in units of surface area, whatever they may mean.

The metabolic rate of female rats as a function of age from 77 to 1000 day could be summarized (with a standard deviation between prediction and observation of only  $\pm 2.2$  per cent of the mean rate) by the following equation:

$$B = 72.6 W^{3/4} (1 + 0.55e^{-0.014 A} + 0.008e^{0.0034 A})$$

where  $B$  = metabolic rate per rat in kcal. per day,  $W$  = body weight in kg., and  $A$  = age in days.

Dill *et al.* (87) have evaluated the performance of one man in maximal work over a period of 28 years, between the ages of 48 and 66. The highest attainable heart rate declined from 172 to 160 and the maximal oxygen consumption from 45.5 to 35 ml. per kg. per min. The results are complicated by a 10 per cent increase in body weight during the period, and the senior author (subject) reports the admirable intention of reducing variability by reducing the weight.

*Tumors and metabolic rate.*—The effects of tumors on energy metabolism present some interesting problems. Benign tumors can grow large enough to become a considerable part of the total mass of an animal, and therefore a study of the metabolic rate of rats with big benign tumors provides information about the effect of a change of body mass on the metabolic rate.

The statement that the metabolic rate of an animal is the weighted sum of the metabolic rates of its various tissues is a tautology which does not pro-

vide new information. But the recognition by Field *et al.* (40) that the metabolic rate of animals can be calculated as the weighted sum of the metabolic rates of excised tissues measured *in vitro* is valuable because it indicates that the metabolic rate measured *in vitro* is a valid index for the metabolic rate of the tissues *in vivo*.

Kleiber & Chernikoff (88) measured the metabolic rates of rats with spontaneous benign breast tumors, one of which amounted to two-thirds of the mass of the rat without tumor. The tumor-bearing rats had a higher metabolic rate per unit of body weight than non-tumor-bearing rats of the same age. The *in vitro* rate of oxygen consumption of tumor tissue, however, was rather low ( $QO_2 = 2$  ml.  $O_2$  per hr. per gm. dry wt.).

The metabolic rate of a rat after surgical removal of the tumor, furthermore, was still abnormally high. The authors concluded that the elevation of the metabolic rate of rats with spontaneous breast tumors was neither the result of an excessive metabolic rate of the tumor tissue nor of a direct stimulation of the metabolism of non-tumor tissue by the tumor. Presumably those conditions which favored the growth of tumors also increase the metabolic rate of the non-tumor tissues and continued to produce a high metabolic rate even after the removal of the tumor.

On the other hand, Mayer & Zomzely (89) reported on obesity in mice with adrenotropic tumors. If tumor-bearing mice were adrenalectomized, then the obesity rapidly disappeared.

*Obesity.*—Interest in obesity continues to increase, and the problem of the regulation of food intake has reached the point at which we can apply the aphorism Szent-Györgyi first coined in speaking of muscle physiology, "The more we know about it the less we understand it."

Although obesity clearly results from an intake of food energy in excess of requirements, Mayer (90) offers an extensive classification of various types of obesity. He has introduced the basic distinction between two types. One is "regulatory obesity", where the primary impairment is the central mechanism controlling food intake; the other is "metabolic" obesity where the lesion is an inborn or acquired error in the metabolism of other tissues. Animals with the latter form of obesity often have a high proportion of body fat without being heavier than their nonobese controls, a condition sometimes surrealistically termed "non-obese obesity".

A great deal of research has been conducted on the metabolic differences between obese and nonobese animals in several species. This material lies beyond the scope of this review, but attention is drawn to a review by Fredrickson & Gordon (91) and to the proceedings of the Brook-Lodge Conference on Energy Balance held in 1959 (92), which will be published in the *American Journal of Clinical Nutrition*.

A key problem in the central regulation of food intake is the nature of the signal by which the body can inform the brain of its nutritional status. Van Itallie (93) points out that there are three possible modalities; chemical, physical, and neural. Of the chemical modalities, Mayer's (90) "glucostatic

theory" has been the most thoroughly investigated, and further impressive evidence for it is reviewed by Mayer (90, 92). The physical modality is represented by the "thermostatic" mechanism proposed by Strominger & Brobeck (94), which is activated by the specific dynamic effect of the ingested food. Finally, a possible nervous signal from fibers among the adipose tissue cells would be an example of the neural modality [Janowitz (95)]. Brobeck (96) considers that no single factor regulates food intake and that it is not possible at the present time to assess quantitatively the significance of possible contributing factors.

There is considerable interest in the relative importance of overeating and underactivity in the etiology of human obesity. Stunkard (97) reports that obese women are less active than normal women but that obese men are as active as normals. He also reports "eating jags" in depression. Johnson *et al.* (98) similarly report that in obese high school girls, inactivity is more pronounced than overeating.

*Food energy.*—In contrast to the efforts of medical researchers directed at the prevention of obesity, workers on agricultural research are concerned with achieving the greatest efficiency of weight gain in farm animals. The Danish National Bureau for Animal Husbandry, the European Association for Animal Production, and the Food and Agriculture Organization of the U.N. (F.A.O.) organized a symposium on energy metabolism at Copenhagen in 1958. The symposium dealt mainly with methods for measuring the respiratory exchange (indirect calorimetry) in farm animals.

In his opening lecture, the senior representative of research in animal energetics, H. Möllgaard (99), listed as a major problem the determination of the nutritive value of feeding stuffs and the appropriation of a single unit to express this value quantitatively.

A year earlier, Kleiber (100) concluded, "The calorie as a unit for measuring chemical energy is suitable for expressing the level of animal feed requirement and for measuring the animal's performance as a food utilizer. Calories do not reveal the nature of specific feed effects. For feed evaluation, the energy content of feeds is inadequate."

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# TRANSPORT THROUGH BIOLOGICAL MEMBRANES<sup>1</sup>

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Only papers published in the period from January 1958 to January 1960 were surveyed for this review. Because of the weight of numbers and the many other competent reviews on topics directly or indirectly related to transport, only a selected and relatively small number of publications are included. In most instances, articles were not cited if they appeared in earlier reviews. The final selection reflects the interests and prejudices of the reviewer and is presented from a partisan viewpoint. Able presentations of the opposing views on the redox pump theory of ion transport by Conway and the modified fixed charge theory by Harris appeared in the *Proceedings* of the Premier Colloque de Biologie de Saclay held in July 1958 (214).

Topics omitted almost in entirety are: renal transport mechanisms, capillary permeability, the electrophysiology of the nervous system and heart, and ion transport in salivary glands, the eye, the placenta, micro-organisms, subcellular particles, and plants. Books relating to transport are: Engström & Finean (75), Ernst (76), and Troshin (273).

## PHYSICAL CHEMISTRY

*Thermodynamical considerations.*—A wealth of information on the physical-chemical properties of ions, solutions of electrolytes and of nonelectrolytes, and ion exchange resins and membranes has been provided in a series of reviews [Poirier (211); Rushbrooke (236); Walton (286); Shedlovsky (248); Rowlinson (233); Argersinger (6); Stern & Amis (261)].

An important development has been the application of the theory of irreversible thermodynamics to biological systems because it explicitly identifies the forces in a transport process. The theoretical and experimental value of this theory in analyzing the events in transport was illustrated by Durbin *et al.* (68). Nims (200) described the application of the principles of irreversible thermodynamics to the transit of water and neutral solutes through membranes. The properties of osmotic and diffusion flow and the sources of the differences in osmotic vs. tracer diffusion-transfer coefficients were carefully considered. The theory itself is based on the Onsager reciprocity relations, the experimental verification of which has been reported for ternary isothermal diffusion [Miller (186); Dunlop & Gosting (67)]. Miller (186) pointed out that this verification establishes that the chemical potential gradients rather than the concentration gradients are the true driving forces for isothermal diffusion. An extended phenomenological treatment of thermal aspects in transport was reported by Haase (97, 98).

<sup>1</sup> The following abbreviations are among those used in this review: ADH (anti-diuretic hormone); DNP (dinitrophenol); p.d. (potential difference).

Scheer (241) restated the problem of the thermodynamic definition of active transport in terms of entropy changes (irreversible processes) and defined two basic types of active transport, designating them as coupled and forced transport. The first type derives its energy from another transport process, while the second is directly dependent on energy-yielding chemical reactions.

*Diffusion through membranes.*—The rational analysis of transfers through membranes begins with an understanding of the properties of the passenger. Electrolytes may be characterized grossly in terms of size (naked and hydrated), charge, and degree of dissociation. Stern & Amis (261) emphasized that ionic radius is an operational concept which has definite meaning only for specified experimental conditions and often depends on the method of calculation. The operational approach to ionic solvation is also implicit in Nightingale's phenomenological-thermodynamical extension of Robinson & Stoke's empirical corrections to the Stoke's diffusion radii (199). He estimated effective hydrated radii by assuming that the tetra-alkylammonium ions are unhydrated, and listed pairs of values for almost all the ions of physiological interest. In biological systems the "wateriness" of the membrane will have a strong influence on the degree of ionic solvation and consequently on the relative membrane diffusion coefficients of analogous ionic species. The lighter ions should be larger in size than the heavier ions in transit through media of high dielectric constant; the reverse should hold in media of low dielectric constant.

The chemical composition of the membrane, the surface charge, and the pore geometry will play critical roles in its selectivity properties. Patlak (208) presented an analysis of diffusion of neutral solutes through pores backed by a large open region, a situation analogous to a cell membrane with the cell contents representing the open area. His solution, however, may be of limited physiological value since it concerns only diffusion through homogeneous water-filled pores.

*Hydrodynamic and diffusion flow across membranes.*—The controversy on the mechanism of solvent flow across membrane boundaries has been resolved on theoretical and experimental grounds. In the ideal case (solute mobility across the boundary is zero), hydrodynamic flow occurs as a direct consequence of the osmotic pressure generated by the impermeant solute. At the other extreme (no restraint to solute penetration), interdiffusion in proportion to the gradient of solvent activity constitutes the primary mechanism for solvent transfer. Most living membranes impose a substantial but not infinite resistance to solute flow. One of the interesting possibilities in "leaky" membranes is spontaneous flow of water against its activity gradient [by Scheer's (241) classification, "coupled" transport]. Meschia & Setnikar (184) studied the magnitude and direction of net flow of water across a colloid membrane permeable to the solvent and to some neutral solutes. For a given osmolar gradient, they found that the rate of solvent flow increased



with the molecular volume of the solute and that in the presence of two or more solutes of differing molecular volumes, water may be transported against its activity gradient. Robbins & Mauro (227) showed that with hemoglobin, inulin, or glucose solutes and counterbalancing hydrostatic pressures across collodion membranes of graded porosities, the calculated diffusion fluxes were 1/36 to 1/730 of the observed hydrodynamic flows. Consequently, at equivalent effective osmotic and hydrostatic pressures, diffusion flow is a negligible component of the total flux.

*Ion-exchange membranes and diffusion.*—The ion-exchange membrane has been the most popular inanimate experimental model of living membranes. The Teorell-Meyer-Sievers theory predicts that the interactions between the ions in solution and the fixed charge sites will yield two Donnan potentials at the boundaries and a diffusion potential across the membrane. Mauro & Finkelstein's measurements of the concentration and potential gradient profiles in a multicompartiment polyelectrolyte cell gave striking support to the theory (179). Another model, an ion-permeable, intranon-permutating membrane,<sup>2</sup> was studied by Kuhn *et al.* (150). In this system the orientation and magnitude of the potential difference depended on the side from which the pores were filled with two different solutions (specifically 0.1 M NaCl:0.0001 M KCl on side 1 and 0.0001 M NaCl:0.1 M KCl on side 2). In the studies cited, the assumption of homogeneity in the activity and distribution of fixed charge sites did not introduce any serious deviations from theory. Cruickshank & Meares (49), however, obtained evidence that the activities of resin exchange sites vary, as does their accessibility to the penetrating ions. Also, the accessibility of a given site may depend on relative ionic size since  $K^+/Cs^+$  exchange did not effect a change in resin volume, whereas  $K^+/H^+$  exchange caused the resin to shrink. A theory of ion transport across charged membranes, formulated by Spiegler (258), described transport processes arising from hydrostatic pressure, electrical, and osmotic forces in terms of the volume concentrations of ions, water, and membrane phase and the frictional coefficients between these components. Meares (181) derived expressions for the ratios of the unidirectional fluxes of cations and anions across a cation-exchange membrane during the passage of electrical current by an irreversible thermodynamical treatment based on Spiegler's frictional model.

Studies on concentration dependence in transport across ion-exchange membranes have yielded interesting results. Kressman & Tye (148) focused their attention on the relationship between the mobility of the gegenion in the membrane and the concentrations of the solutions on either side of the membrane and concluded that the gegenion transport number is dependent on either the solution of origin or on the solution of receipt but never on

<sup>2</sup> An intranonpermutating membrane is a membrane with small pores that allow only single file passage of ions.



both. Meares & Ussing (182) measured the flux ratio for radioactive  $\text{Na}^+$  and  $\text{Cl}^+$  and found good agreement between the theoretical passive diffusion flux ratios and the measured values when concentration gradients provided the transport force. A disturbing finding, however, in view of Miller's evidence that the chemical potential provides the driving force for diffusion processes (186), was the lesser accuracy obtained when they included the activity coefficients in the calculation of the flux ratio.

Passive ion transport through charged membranes does not always conform to the predictions of a flux ratio equation when an electrical gradient provides the driving force. Meares & Ussing (183) measured the unidirectional tracer fluxes across a cation exchange membrane during the passage of constant currents. Good agreement was obtained between theory and measurement for the gegenions but not for the nebenions when the equations were based on self-diffusion coefficients and the over-all rate of electro-osmotic flow. Since the nebenion conductance appeared to be a function of the potential gradient, the authors concluded that flux ratio equations must be used with caution when there is a substantial contribution to the fluxes from an electric current. Teorell (265, 266) described rhythmic variations of the transmembrane potential when simultaneous electrical and chemical gradients were used to drive the system, which he ascribed to the interplay of electro-osmotic and hydrodynamic flows. He suggested that the applied electrokinetic concepts may be important in understanding the behavior of excitable living cells.

Caution must be exercised, however, in accepting water-filled ion-exchange resins as reliable models of biological membranes. Charged, inanimate membranes are relatively homogeneous over their surface and in cross section. Living membranes are far more complex, probably are heterogeneous over their surfaces and in cross section (aqueous and lipid phases are both present), and may be capable of rapid changes in fixed charge density and distribution.

*Transfer rates and permeability coefficients.*—It must be borne in mind that diffusion and hydrodynamic permeability coefficients are independent proportionality factors [Nims (200); Robbins & Mauro (227)]. Dick (62) failed to appreciate this fact when he measured the volume changes in cultured chick heart fibroblasts and estimated the diffusion coefficient of water in the cytoplasm from the hydrodynamic (osmotic pressure) permeability coefficient. Leaf (158) described a tracer method for estimating the diffusion permeability coefficients of the individual surfaces of a compound cellular structure. The anatomical identification of the diffusion paths and boundaries in such a system is still uncertain, however, since cytoplasmic mixing terms [Patlak (208)] and intercellular channels may contribute to the observed transmucosal rate of transfer.

#### WATER TRANSPORT AND OSMOTIC EQUILIBRIUM

*Active transport of water.*—The active transport of water as a primary event ["forced" transport in the Scheer (241) classification] does not appear

to occur in biological systems. The finding by Edelman *et al.* (74) that plasma osmolality correlated closely with the ratio of exchangeable sodium plus exchangeable potassium per liter of total body water in man strongly supports the concept of passive distribution of water throughout most if not all of the fluid compartments. Moreover, the activity of water in plasma and in tissues does not differ significantly when suitable precautions are taken to avoid autolysis which occurs rapidly in tissues even at 0°C. [Buckley *et al.* (30)]. Boiled, as well as frozen-thawed, extracts of mammalian tissues, including liver and renal cortex, were found to be iso-osmotic with plasma [Appelboom *et al.* (5)]. To circumvent the problem of autolysis, Maffly & Leaf (174) constructed melting point curves for tissues frozen in liquid nitrogen and showed that rat muscle, liver, heart, and brain were in osmotic equilibrium with plasma. Swelling of tissue slices, which was attributed to active water transport in earlier studies, has been adequately explained by solute penetration into the slice. Choline and sulfate ions penetrate slices of renal cortex readily at 37°C. [Maizels & Remington (175)], Na<sup>+</sup> and Cl<sup>-</sup> invade liver slices in proportion to the initial stage of swelling [Heckmann & Parsons (107)], and the water content of the rat diaphragm varies linearly with the sum of tissue Na<sup>+</sup> plus K<sup>+</sup> on incubation in sucrose solutions [Rixon & Stevenson (226)]. Moreover, the quantitative deviations from the simple Van't Hoff predictions in the swelling of erythrocytes at various plasma osmolalities were logically explained by the change in the osmotic coefficient of hemoglobin as a function of hemoglobin concentration [Dick & Lowenstein (63); Dick (60); Williams *et al.* (291)]. Finkinhstein (79), apparently unaware of the data on solute transfers during anaerobic incubation of liver, muscle, or kidney, concluded that anaerobic tissue swelling was evidence of active extrusion of cell water attributable to elongation and contraction of cytoplasmic protein micelles.

*Factors governing water flux.*—Dick (61) concluded that instantaneous mixing of water in cell cytoplasm does not occur, because in a variety of cells the permeability coefficients (osmotic) correlated closely with the surface:volume ratios. Villegas *et al.* (278) applied a modified Pappenheimer method in a comparative study of beef and dog erythrocytes. From the tracer rate constants and the hydrodynamic (osmotic gradient) permeability coefficients, they estimated an equivalent pore radius of 4.1 and 7.4 Å for beef and dog erythrocytes, respectively. These values, however, are approximations based on the assumption that the cell membranes were completely impervious to solute.

An important factor to be considered in interpreting transport data is the degree of heterogeneity in the cell population under study. Jaffé *et al.* (126) demonstrated that young erythrocytes are more resistant to hypotonic lysis than old ones and that inosine offers greater protection from osmotic lysis to young cells than to all the erythrocytes combined.

The role of solute transfer in water transport was clearly demonstrated by the observations of Windhager and co-workers (294) that net water flux was a linear function of luminal NaCl concentration in single proximal tu-

bules of the necturus kidney. Whittembury *et al.* (289) calculated a hydrodynamic permeability coefficient of  $0.15 \times 10^{-8}$  ml.  $\cdot$  cm. $^{-2}$   $\cdot$  sec. $^{-1}$   $\cdot$  cm. $^{-1}$  H<sub>2</sub>O, assuming a Staverman coefficient of 1.0 for mannitol, in the same system. Both ouabain and dinitrophenol, inhibitors which are known to depress or block active ion transport, reduced the net water flux considerably in this system [Schatzmann (240)]. In studies on the isolated rat jejunum, Parsons & Wingate (207) found that water moved against its activity gradient and in the direction of net solute flux, provided that glucose was available as substrate in isotonic NaCl. Substitution of LiCl for NaCl stopped net water flux, which supports their hypothesis of electro-osmotic flow. Cooperstein & Brockman (40) noted that the direction and magnitude of net water flux were determined by the combined rates of Na<sup>+</sup> and HCO<sub>3</sub><sup>-</sup> transport across the large bowel in dogs.

*Antidiuretic hormone.*—Pituitary extract increased the net flow of water across the toad bladder in the presence of an osmotic gradient [Bentley (13, 14)]. In the absence of ADH, Ca<sup>++</sup> maintained the normal impermeability of the bladder to water and augmented the response to pituitrin. Sodium on the epithelial side of the toad bladder and potassium on the serosal side supported the ADH response, while anaerobiosis and metabolic inhibitors (iodoacetate, malonate, cyanide, and dinitrophenol) blocked the response. These observations do not, however, mean that the effect of ADH on the permeability coefficient is metabolically mediated.

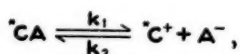
In mammals, the kidney may not be the only site of action of ADH in view of the finding by Fishman (80) that vasopressin diminished the half-time of <sup>24</sup>Na-exchange in cerebrospinal fluid in anesthetized dogs; corticosteroids, norepinephrine, and insulin were without effect. Species differences also affect the hormonal regulation of water permeability, as shown by Morel and co-workers (190). Oxytocin was more effective than vasopressin in stimulating Na<sup>+</sup> and water transport across the skin of *Rana esculenta* and *Rana temporaria*, but the two hormones were equally effective in *Bufo bufo*. Ginetzinsky (90) proposed that ADH increases the permeability of renal collecting ducts to water by its effect on hyaluronidase activity. He observed that hyaluronic acid disappeared from the basement membrane of the collecting ducts when ADH was administered to rats. Schmidt's data did not support Ginetzinsky's concept, since the speed at which KCl decreased the membrane potential of the frog sciatic nerve was unchanged by the application of hyaluronidase (242). Thorn's review (269) is worthy of study.

#### ION TRANSPORT

Comprehensive and competent reviews have covered many aspects of ion transport, including electrophysiology of muscle and nerve, theories of transport and ionic equilibria in animal tissues and micro-organisms [Shanes (246, 247); Katz (134, 135); Cranefield & Hoffman (47); Kuffler (149); Glynn (91); Berliner (16); Leaf (159); Rothstein (230); Ussing *et al.* (276, 276a)].

*Fixed charge vs. membrane transport theory.*—Most of the current evidence firmly supports the membrane locus theory of transport as a unifying concept in describing the mechanism of ionic distribution and equilibria across cell membranes, electrolyte and water transport by secretory cells, and the electrophysiological events in excitable cells. The alternative view that ionic gradients in nonsecreting cells result from selective binding of  $K^+$  to an ordered macromolecular cytoplasmic phase rich in fixed negative charges is, in essence, a restatement of the Ling fixed-charge hypothesis [Lester & Hechter (166); Simon *et al.* (251); Frater *et al.* (82); Simon (250); Ernst & Hajnal (77); Bozler *et al.* (24)]. According to Lester & Hechter (166), azide and dinitrophenol inhibited the uptake of  $Rb^+$  by *Neurospora crassa* but did not release cellular  $Rb^+$ , and  $Rb^+$  and  $K^+$  did not exchange in germinating conidia, even in the absence of metabolic inhibitors. They concluded that  $Rb^+$  and  $K^+$  are accumulated by a specific cytoplasmic binding system. The membrane theory, however, could equally well account for their observations since the movement of  $Rb^+$  and  $K^+$  is expected to depend both on the specific ionic conductances in the membrane and the total driving forces (i.e., the electrochemical gradients), which Lester & Hechter did not measure. Simon *et al.* (251) found that the volumes of distribution of  $Na^+$  and  $Li^+$  in muscle were indistinguishable and that the amount of  $Na^+$  in the slow fraction of  $Na^+$  efflux was increased by  $Li^+$  loading, yet failed to recognize that these data are inconsistent with their own theory. If  $K^+$  is preferred to  $Na^+$  as the bound ion, then  $Na^+$  should be preferred to  $Li^+$  and have the larger volume of distribution. If both ions are excluded from the "ordered" phase, there is no reason for the slow  $Na^+$  component to increase in amount. Moreover, Keynes & Swan (138) verified that the extrusion of  $Na^+$  is effectively blocked in frog muscle soaked in  $K^+$ -free media and that the mechanism responsible for generating the action potential does not discriminate between  $Na^+$  and  $Li^+$ , but that the extrusion of  $Li^+$  is only 1/10 to 1/25 as fast as  $Na^+$  extrusion. These results are consistent with the membrane theory since the active transport mechanism is probably located in a region of low dielectric where the smaller naked  $Li^+$  can be expected to bind more securely than  $Na^+$  to the "carrier" and consequently would be extruded at a slower rate. The evidence for divalent ion binding to cytoplasmic constituents offered by Frater *et al.* (82) does not provide a sound basis for assuming that monovalent ions would show similar binding properties since a doubling of charge would greatly increase the strength of binding. Ernst & Hajnal (77), by perfusing frog skeletal muscle with  $K^+$ -enriched Ringer's solution, found that a tenfold increase in the concentration of  $K^+$  in the perfusate (i.e., 4 to 40 m.eq. per l.) produced only a 10 per cent increase in muscle  $K^+$  and that the added  $K^+$  washed out readily. They concluded that muscle  $K^+$  is bound selectively to specific subcellular components. However, they did not appreciate that in an anion-impermeable system, the principle of electroneutrality requires the extrusion of one equivalent of  $Na^+$  for each equivalent of  $K^+$  entering the cell and as a

consequence  $K^+$  accumulation is limited by the capacity of the  $Na^+$  pump. The early release of newly added  $^{42}K$  into the washout medium can be explained by a failure to reach diffusion equilibrium (nonuniform distribution of  $^{42}K$  between the interstitial and intracellular phases). Bozler *et al.* (24), in studying  $^{42}K$ -exchange in frog gastric smooth muscle, noted a rapid and a slow exchange component and concluded that the slow component reflected intracellular adsorption of  $K^+$ . This and many other such studies in the past suffer from the misconception that ion binding may be inferred from rates of exchange and that the binding process necessarily slows the rate of release. In a dissociation reaction:



the strength of binding is given by the ratio of  $k_1/k_2$  whereas the rate of escape of the isotope  $^*C$  from a cell depends only on  $k_1$  in the presence of an infinite sink. For membranes of low conductance, the transmembrane flux may be slower than the exchange of an ion on fixed charge sites. Moreover, other plausible explanations are at hand to explain multicomponent efflux curves in multicellular systems. For one thing the membranes of the endoplasmic reticulum, mitochondria, and nuclei may present diffusion boundaries. For another, cell populations are apt to be heterogeneous so that fast and slow components may simply represent subpopulations of cells with varying membrane permeability coefficients for the species in question. Either of these possibilities may explain the observations of Rayner & Weatherall (218) that  $^{42}K$ -exchange in contracting rabbit auricle segments required at least two exponential terms for its description and that the initial component was faster in contracting auricles than in quiet ones. Cellular heterogeneity has been clearly revealed in studies on erythrocytes. Prankerd (213) separated young and old erythrocytes by centrifugation in 30 per cent albumin solutions and found no differences in phosphate ester content but more  $K^+$  and less  $Na^+$  in young cells. Erythrocytes taken from the top layers of a cell column had higher  $K^+$  concentrations and faster  $^{42}K$ -exchange rates than those taken from the middle of the column [Joyce (132)]. Maizels & Remington (176) stated that human red cells contain 2 to 3 m.eq. per l. of  $Na^+$  which exchanges very rapidly with radiosodium or with  $K^+$  or  $Li^+$  in an external medium and concluded that this fraction is closely associated with the cell surface; deviations from first-order kinetics in the major component of the exchange curve were attributed to heterogeneity of the cell population.

Evidence in support of the membrane theory was offered by Green *et al.* (93) and by Watanabe (284). Addition of rabbit antibody plus complement to mouse ascites tumor cells resulted in the rapid disappearance of the ionic concentration gradients but no loss of intracellular proteins or nucleotides (93). In single amphibian muscle fibers, longitudinal currents inside the

fiber initiated contraction only at very high currents compared with those required to depolarize the membrane (284). Ussing (276) summarized some of the evidence in favor of the membrane transport theory. The following arguments may also be offered. (a) The membrane theory provides a consistent and coherent explanation of ion transport in all cells, conducting or nonconducting, secreting or nonsecreting, while the fixed charge theory fails to account for secretory processes without cellular accumulation [e.g.,  $\text{Na}^+$  transport by cells low in  $\text{Na}^+$  (kidney, frog skin, toad bladder),  $\text{H}^+$  transport by cells low in  $\text{H}^+$  (gastric mucosa)]. (b) The membrane theory relates the fluxes and forces quantitatively, whereas the fixed charge theory until now has been expressed qualitatively. (c) The argument by Simon (250) that the fixed charge theory is less costly thermodynamically is unsound: the minimum energy cost to the cell is the product of the molal free energy change and the net flux, regardless of the mechanism for creating the concentration gradients.

*Membrane theories.*—The details of the microscopic events in membrane transport remain obscure. Koblick (144) proposed an enzyme-dependent ion-exchange model to account specifically for active  $\text{Na}^+$  transport. The equation derived to relate net  $\text{Na}^+$  flux to  $\text{Na}^+$  concentration gradients agrees qualitatively with data on isolated frog skin. A more mechanically complete scheme, offered by Miller (187), pictures the diffusion barrier at the cell surface as a dense gellular layer. Enzyme action presumably induces changes in the number of osmotically active groups in the gellular barrier, which in turn bring about a reciprocating uptake and expulsion of solution by the membrane. The driving mechanism is the alternate movement of water into and out of the membrane gel phase (an osmotic pump). It is difficult, however, to see how this theory could account for  $\text{Na}^+$  transport without appreciable water transport such as occurs in the toad bladder. Zierler (297) posited a dynamic model in which diffusion sites appear and disappear in waves rippling over the cell surface, based on his observation that metabolic inhibitors markedly increase passive aldolase efflux. According to Wilbrandt and co-workers (35, 262, 290), active inorganic cations  $M_a$  combine in complex chelates with a fixed membrane receptor  $R$  and a labile carrier  $A$  to form the specific transporting complex  $A \cdot R \cdot M_a$ . In intact rats, potassium strophanthoside decreased and adrenal extracts increased urinary sodium excretion; in adrenalectomized rats the effects were reversed. Wilbrandt and his associates proposed that the glycoside competitively displaces corticosteroid as an ion carrier chelate in the membrane. In Ascheim's theory (7, 8), the primary event during nerve excitation is the sorption and desorption of an ionic layer on the membrane surface. He assumed that the binding layer is in equilibrium with the cytoplasmic phase during rest and equilibrates with the external medium in a single discharge. A critical test of this theory, however, has not yet been devised. The possible role of cellular concentration mechanisms in transcellular transfers was emphasized by

Oxender & Christensen (202). Using a membrane formed by depositing ascites tumor cells on a millipore filter, they found that amino acids were concentrated away from the side to which pyridoxal was added and  $K^+$  was concentrated toward the side spiked with glycine.

*Experimental characterization of the membrane.*—The cell membrane appears to be a highly organized structure, 50 to 150 Å thick, consisting of lipid and protein with charged sites distributed in aqueous and nonaqueous channels. The active transport mechanism derives its energy from metabolic processes and presumptively achieves its specificity from the binding constants of the interaction between ion and charged site, the dielectric constant of the medium in the channel, the hydrated and unhydrated radii of the passenger ion, and the dimensions of the channels for passive and active transit. If the passive component of the flux involves ion exchange (i.e., the "downhill" ion replacing the "uphill" ion at the carrier site), the shunting channel and active transport channel would be the same and the system would behave like a series of "long pores" [see Hodgkin & Keynes (114)].

Conway & Duggan (39) evaluated the binding characteristics of the "carrier" in the yeast cell membrane by a Lineweaver-Burk plot of the rate of uptake of ion as a function of ion concentration. The alkali metal ions were calculated to have relative affinities for the carrier of 100:42:7:3.8:0.5 for  $K^+$ ,  $Rb^+$ ,  $Cs^+$ ,  $Na^+$ , and  $Li^+$ . This corresponds to order 4 in the Rudin-Eisenman (234) analysis of the effect of increasing electrostatic field strength of the fixed anion on the binding affinity of the cation. Rothstein & Bruce (231) observed that the effect of  $K^+$  concentration on  $K^+$  influx also conformed to the Michaelis-Menten equation in fermenting yeast cells. In frog sartorius incubated *in vitro*, replacement of  $Na^+$  in the external medium by choline or  $Li^+$  reversibly reduced  $^{22}Na$  efflux to half the control value, a result consistent with the Ussing exchange diffusion postulate [Keynes & Swan (137)]. The decrease in  $Na^+$  efflux in  $Na^+$ -free solutions disappeared in muscles cooled to below 10°C. or stored in Ringer's solution for more than 12 hr. or in  $K^+$ -Ringer's solution. The authors postulated two pathways for  $Na^+$  efflux: an exchange diffusion mechanism, and an additional diffusion pathway. Kirschner (142) proposed an alternative hypothesis to account for the decrease in  $Na^+$  efflux with a decrease in external  $Na^+$  concentration, which, however, does not differ in essence from the Ussing exchange diffusion postulate. McLennan (173) tried to distinguish between desorption of  $Na^+$  from the cell membrane of rat skeletal muscle and  $Na^+$  transport across the membrane by an analysis of the curve of  $^{22}Na$  washout. He inferred that incubation in  $K_2HPO_4$  increased the rate of desorption without affecting  $Na^+$  movement across the cell membrane and that strophanthin had the reverse effect. The interpretation of tracer diffusion curves, however, is still controversial.

The anionic sites most likely to be involved in cation transport mechanisms are carboxyl, hydroxyl, sulfhydryl, and phosphoryl groups. A number of studies were concerned with the role of the latter two groups in cation



transport. Bangham *et al.* (12) concluded from studies on the effects of various cations on the electrophoretic mobility of cells that the dominant group determining the electrokinetic potential was a phosphate on the red cell surface and a carboxylate on the leukocyte surface. The participation of phosphate groups in ion transport was suggested by the finding that exposure of lobster giant axons to phospholipase A or C depressed excitability and impulse conduction much more than equivalent exposure to trypsin and alpha chymotrypsin [Tobias (270)]. Similarly, Nelson (197) stated that disruption of phospholipid by phospholipases applied to the nodal region of isolated myelinated frog axons resulted in a loss of excitability, while proteases had a lesser and delayed effect. Kirschner (141) isolated four phospholipid fractions from swine red cells; in two of the fractions  $\text{Na}^+$  was the predominant cation under all conditions and in the other two fractions the Na:K ratio varied with the extraction procedure.

The participation of membrane-SH groups in ion transport mechanisms has been inferred from the effects of sulfhydryl blocking agents on the electrophysiological properties of nerve. Cysteine reversed the prolongation of the action potential induced by a number of heavy metals in amphibian myelinated nerve fibers and heart muscle [Uchizono & Matsumoto (275); Takahashi *et al.* (264)]. Oxophenarsin decreased the rate of repolarization, whereas mersalyl and cupric aminoacetate depressed the resting potential in the isolated rat diaphragm [Muscholl (194)]. Frog and lobster nerve became inexcitable when treated with *n*-ethyl maleimide and *p*-chloromercuribenzoic acid [Smith (252)]. The participation of -SH groups in ion transport was also suggested by the great increase in  $\text{K}^+$  efflux from yeast cells when they were treated with redox dyes or mercurials [Rothstein & Bruce (232)]. Although the order of activity of heavy metals in releasing red cell  $\text{K}^+$  was  $\text{Pb} > \text{Hg} > \text{Au} > \text{Cu} > \text{Cd} > \text{Zn}$ , Vincent & Blackburn (280) found that the thiol inhibitors sodium iodoacetate, sodium tetrathionate, sodium arsenite and sodium arsenate had no effect on red cell  $\text{K}^+$  loss. It appears unlikely that all of the heavy metals bind to -SH groups, and a variety of other groups may be involved in maintaining the integrity of the cell membrane [Vincent (279)].

*Calcium effects on cell membranes and transport.*—A wealth of data has established the importance of  $\text{Ca}^{++}$  in determining membrane permeability and correlated transport properties. Only brief mention can be made of the many experiments on the effects of  $\text{Ca}^{++}$  and related divalent ions on transport phenomena.

Lowering of  $\text{Ca}^{++}_o$  concentration below 25 mM reduced both the resting and action potentials in lobster giant axons [Dalton (50)]. In the single myelinated nerve fiber of the frog, excess  $\text{Ca}^{++}_o$  resulted in a sharp rise of threshold and slowing of conduction [Hashimura & Wright (105)]. The authors proposed that  $\text{Ca}^{++}_o$  competes for cationic binding sites in the membrane, thereby determining the permeability to sodium. Presumably the action



potential is initiated by withdrawal of  $\text{Ca}^{++}$  from the membrane. Koketsu & Nishi's finding that in frog sartorius,  $\text{Ca}^{++}$  was required for the hydrazinium restoration of the resting potential and action potential in  $\text{Na}^+$ -free solutions supports this postulate (146). Furthermore, the rates of rise and of fall of the action potential in toad muscle fibers were reduced approximately in proportion to the increase in  $\text{Ca}^{++}_o$  [Ishiko & Sato (124)]. Niedergerke (198) also posited a reversible competition between  $\text{Na}^+$  and  $\text{Ca}^{++}$  for a membrane combining site because he noted that removal of  $\text{Na}^+$  facilitated  $\text{Ca}^{++}$  uptake in the frog ventricle. In contracting rabbit atria, lowering  $\text{Ca}^{++}_o$  blocked the depressing effect on  $\text{K}^+$  flux of low  $\text{Na}^+_o$ , suggesting that  $\text{Ca}^{++}$  determines the magnitude of coupling of the  $\text{Na}^+$  and  $\text{K}^+$  fluxes [Holland *et al.* (119)].

These effects, however, may also involve the entry of  $\text{Ca}^{++}$  into the muscle cell since  $\text{Ca}^{++}$  may act as the link between excitation and contraction [Frank (81)]. Frog skeletal muscle, when quiescent, exchanged only 26 per cent of its  $\text{Ca}^{++}$  in eight hours, but at contraction rates of 15 per min. achieved 100 per cent exchange in less than seven hours [Cosmos (41)]. Rapid stimulation of isolated rabbit atria (1200 per min.) increased  $^{45}\text{Ca}$  influx about 45 per cent and  $^{36}\text{Cl}$  influx about 86 per cent; ouabain also increased  $^{45}\text{Ca}$  and  $^{36}\text{Cl}$  influx rates, suggesting that contraction may act on the passive permeability component [Sekul & Holland (120, 245)].

The permeability of the red cell to  $\text{K}^+$  appears to be determined largely by the activity of  $\text{Ca}^{++}$  in the medium. The addition of ethylenediaminetetraacetic acid, sodium hexametaphosphate or sodium oxalate blocked the enhanced  $\text{K}^+$  efflux in erythrocytes in which glycolysis had been suppressed by metabolic inhibitors [Gardos (89)]. Uptake of  $\text{K}^+$  by cold-stored red cells was impaired at high  $\text{Ca}^{++}_o$  and by ouabain, but in contrast to its effects on the heart, ouabain does not alter  $\text{Ca}^{++}$  uptake, which contradicts the idea that the primary glycosidal effect is to increase the permeability of the cell membrane to  $\text{Ca}^{++}$  [Kahn (133)]. Bolingbroke & Maizels (22) incubated human erythrocytes in lactose, thereby increasing cation permeability. Of all of the alkaline earth metals, only  $\text{Ca}^{++}$  proved capable of restoring the normal low permeability to  $\text{K}^+$ ,  $\text{Na}^+$ , and  $\text{Li}^+$  in this preparation. It was suggested that membrane-bound  $\text{Ca}^{++}$  is an essential component in the normal poorly permeable state of erythrocytes. But if this is so, why did inactivation of  $\text{Ca}^{++}$  by chelating agents reduce  $\text{K}^+$  efflux (89)?

The active transport of  $\text{Ca}^{++}$  across the intestinal mucosa was postulated by Schachter & Rosen (238) inasmuch as  $^{45}\text{Ca}$  accumulated against a concentration gradient on the serosal side of the guinea pig gut sac and  $\text{Mg}^{++}$ , metabolic inhibitors, and vitamin-D deprivation reduced  $^{45}\text{Ca}$  transport. The failure of Schachter & Rosen to measure the electrical gradients and to correct for solvent drag, however, leaves the issue of active transport of  $\text{Ca}^{++}$  in this system unsettled. The possible role of metabolic factors in  $\text{Ca}^{++}$  uptake was suggested by Thomason & Schofield (267) who found that ascites tumor cells and peritoneal fluid *in situ* showed the same specific activity

four minutes after injection of  $^{45}\text{Ca}$ , but no uptake of  $^{45}\text{Ca}$  was detected in ascites cells incubated in ascitic fluid *in vitro*.

*Ionic gradients and membrane potentials.*—Hodgkin & Horowicz (112, 113) provided strong support for the membrane transport theory of ionic accumulation and excitation. By isolating single muscle fibers from the frog's semitendinosus, they avoided the difficulties of interfiber diffusion and of heterogeneity which complicate studies on multicellular preparations. That fibers preloaded with  $^{24}\text{Na}$  and  $^{42}\text{K}$  lost the radioion at a single exponential rate over a period of hours contradicts the concept of a multiphasic distribution of  $\text{Na}^+$  and  $\text{K}^+$  in any one cell. Conduction of impulses was associated with movements of  $\text{Na}^+$  and  $\text{K}^+$  down their concentration gradients. Moreover, the membrane potential varied in the manner of a  $\text{K}^+$  electrode when  $\text{K}^+_0$  and  $\text{Cl}^-_0$  were varied reciprocally to maintain a constant product ( $\text{K}^+_0 \times \text{Cl}^-_0$ ), and in  $\text{Cl}^-$ -free solutions the potential difference followed the Nernst equation prediction. At constant  $\text{K}^+_0$ , changes in  $\text{Cl}^-_0$  produced large transient changes in potential difference, lasting 10 to 60 min. in the direction expected for a chloride electrode. These results favor the Boyle-Conway theory of a Donnan distribution system for  $\text{K}^+$  and  $\text{Cl}^-$  across the muscle cell membrane. In the resting membrane the current is carried predominantly by  $\text{K}^+$  and  $\text{Cl}^-$ , and their relative contribution to the membrane potential depends on the direction of  $\text{K}^+$  flow. According to the Nernst equation the resting potential should change by 0.32 per cent per degree C. Creese *et al.* (48) saw no change in the mean transmembrane potential of the rat diaphragm between 39 and 15°C. In sartorius muscles of *Hyla coerulea* there was a slight increase in potential difference from 0 to 38°C. and a moderate decrease between 38 and 45°C. [MacFarlane & Meares (171)]. These deviations from the predictions of the Nernst equation may mean that the membrane permeability coefficients for  $\text{K}^+$  and  $\text{Cl}^-$  are temperature dependent or that temperature changes may have differential effects on specific ionic activities in the cytoplasm. Findings in nerve fibers and cardiac and smooth muscle also favor the Boyle-Conway theory. Replacement of  $\text{Cl}^-_0$  depolarized myelinated nerve fibers, most noticeably at  $\text{K}^+_0 = 10 \text{ mM}$  [Schmidt & Stämpfli (243)]. Vaughn Williams (277) reported that the resting potential in the isolated rabbit atrium had a slope of  $-56 \pm 1.8 \text{ mv.}$  per tenfold change in  $\text{K}^+_0$  which is consistent with a very low  $\text{Cl}^-$  permeability. Burnstock & Straub (32) obtained a 26 mv. depolarization per tenfold change in external  $\text{KCl}$  concentration in smooth muscle; isotonic  $\text{K}_2\text{SO}_4$  produced a 55 mv. depolarization, and the p.d. vs.  $\log \text{Cl}^-_0$  slope was 41 mv., which agrees qualitatively with the prediction of Hodgkin & Horowicz (113) that in a  $\text{K}^+$  and  $\text{Cl}^-$  permeable system the resting potential  $v$  will be related to  $\text{K}_0$  and  $\text{Cl}_0$  by the equation:

$$\left( \frac{\partial v}{\partial \log K_0} \right)_{\text{Cl}_0} - \left( \frac{\partial v}{\partial \log \text{Cl}_0} \right)_{K_0} = 58$$

provided that the current flow through the resting membrane is given by the sum of the net flux of  $K^+$  and  $Cl^-$  and that  $K_i$  and  $Cl_i$  are invariant. The single electroplate of the ray also conforms to this prediction since Brock & Eccles (29) observed that above a base-line level the resting potential difference varied linearly with  $\log K^+_o$  with a gradient of 58 mv. per tenfold increase in  $K^+_o$ . Chloride does, however, participate in the action potential since the electroplates were inexcitable in solutions free of  $Ca^{++}$  or  $Cl^-$ .

Discrepancies between ionic gradients and resting membrane potentials were described by Lüllman (168). In segments of rat diaphragm soaked in saline for three hours, the potential calculated from the measured electrolyte gradients by the Hodgkin-Katz equation deviated progressively from the measured potential difference. As Lüllman pointed out, this probably means that the specific permeability coefficients were changing progressively as the concentration gradients declined. Chalaikhian (36) reviewed some of the evidence for the ionic gradient-membrane theory and defended its basic premises.

*Ion transport and excitation.*—Most of the recent work supports the basic validity and generality of the Hodgkin-Huxley-Katz theory of excitation in nerve and muscle which ascribes depolarization to a sudden increase in membrane  $Na^+$  conductance, followed by an increase in  $K^+$  conductance; recovery is associated with restoration of permeability coefficients and resting ionic gradients. Chloride rather than  $Na^+$  is actively transported from the medium into the clear sap of the internodal cells of the fresh water algae *Chara* against an electrical gradient of 180 mv. according to Gaffey & Mullins (88), while  $K^+$  was in electrochemical equilibrium and  $Na^+$  nearly so, in the sap. Furthermore, depolarization by electrical stimulation induced a transient increase in  $Cl^-$  permeability, indicating the same general properties as in nerve and muscle except that anion- rather than cation-active transport provides the source of the electromotive force.

According to Yamasaki & Narahashi (282), in the giant axon of the cockroach the positive phase of the action potential varied with the  $\log Na^+_o$  as predicted by the sodium conductance theory. In the rat diaphragm, the overshoot of the action potential was also linearly dependent on  $\log Na^+_o$ , with a slope of 59.6 mv. per tenfold change in  $Na^+_o$  [Muscholl (195)]. The rate of rise and amplitude of the action potential also depended on  $Na^+_o$  in sheep and calf ventricles [Délèze (57)]. Moreover, Dodge & Frankenhaeuser (64) obtained evidence of rectification in the membrane of single nodes of Ranvier and argued that the sodium permeability coefficient as described by the constant field equation accounted well for the rectification.

The substitution of guanidine for  $Na^+$  reduced the rate of rise of the action potential to 20 per cent of normal in isolated nodes of Ranvier [Lüttgau (170)]. The guanidine cation apparently has suboptimal conductance characteristics in substituting for  $Na^+$ , whereas the configuration of the action potential of frog spinal ganglion cells was almost the same in  $Na$ -free hy-

drazinium solutions as in normal Ringer's [Koketsu *et al.* (145)]. Although these data appear to fit the hypothesis of an organic cation acting as a substitute for  $\text{Na}^+$  in carrying charge across the membrane, the authors rejected this interpretation. However, even divalent inorganic cations may substitute for  $\text{Na}^+$  in the depolarization process. Impulse conduction in mammalian B and C nerve fibers was blocked in isotonic solutions of  $\text{MgCl}_2$ ,  $\text{CaCl}_2$ ,  $\text{CdCl}_2$ , and  $\text{MgCl}_2$ , but prolonged action potentials of high amplitude were obtained in isotonic  $\text{BaCl}_2$  [Greengard & Straub (94)]. Substantial action potentials were also obtained in crayfish muscles bathed in isotonic  $\text{SrCl}_2$  or  $\text{BaCl}_2$  [Fatt & Ginsborg (78)].

Daniel & Singh (54), however, rejected the sodium current theory of depolarization in smooth muscle because 90 per cent replacement of  $(\text{NaCl})_0$  with choline or sucrose did not reduce the magnitude of either the resting or action potentials in cat myometrial cells. Although the choline chloride results are consistent with the sodium theory, the preservation of the electrical properties in sucrose is difficult to rationalize.

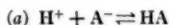
The metabolic events in the membrane during electrical activity were discussed in a number of reports. Abbott, Hill & Howarth (1) measured heat production in the course of single impulses in nonmedullated nerve fibers at  $0^\circ\text{C}$ . An average positive heat production of about  $9 \times 10^{-6}$  cal. per gm. of nerve was obtained during the action potential, followed by a slower heat absorption phase of about  $7 \times 10^{-6}$  cal. per gm. They suggested that part of the heat during an impulse may derive from the decay of the sodium-potassium gradients (i.e. heat of mixing) and part from the chemical reactions which may underlie the permeability change accompanying activity. Schofeniels (244) calculated an activation energy of 21,000 cal. per mole for the rate-limiting step in depolarization in the membrane of the isolated electroplax of *Electrophorus electricus* and favored the Nachmansohn postulate that chemical processes control membrane permeability during excitation. Dettbarn *et al.* (59) cited the eserine reversal of the depolarizing action of pyridine aldoxine dodecylchloride in frog nerve in further support of the chemical theory.

*Anion transport.*—Anion transport in muscle and nerve appears to be passive. If the channels are entirely aqueous and the anion does not interact with the membrane, the mobilities in the membrane should correspond to the equivalent ionic conductances (i.e.,  $\text{Br}^- > \text{I}^- = \text{Cl}^- > \text{F}^-$ ) in the halide ion series. Using a continuous short-circuit technique, Mullins (192) noted that the ratio of the  $^{36}\text{Cl}$  to  $^{131}\text{I}$  permeability rates were 2.5:1 and 1.8:1 in Cl-Ringer's and in 50:50 Cl-I-Ringer's, respectively. He concluded that enough water of hydration is shed in transit to provide penetration rates proportionate to the crystal or naked radius of the ion. The halide ions have radii of 1.29 Å ( $\text{F}^-$ ), 1.81 Å ( $\text{Cl}^-$ ), 1.97 Å ( $\text{Br}^-$ ), and 2.23 Å ( $\text{I}^-$ ). Hutter & Padsha (122) measured total membrane resistance of frog skeletal muscle in Ringer's solutions modified by substituting anions for  $\text{Cl}^-$  and reported relative

resistances of 1.0:1.5:2.0:2.3 for  $\text{Cl}^-$ ,  $\text{Br}^-$ ,  $\text{NO}_3^-$  and  $\text{I}^-$ -Ringer's, respectively. Chloride conductance, therefore, contributes more than half the total conductance. Depolarization, however, need not occur on substituting for  $\text{Cl}^-$  since the analogous ion also reduces  $\text{Cl}^-$  flux. Harris (101) showed that  $\text{Cl}^-$  outflux from frog sartorii is slowed in solutions in which some of the  $\text{Cl}^-$  has been replaced by either  $\text{Br}^-$ ,  $\text{NO}_3^-$ ,  $\text{I}^-$ ,  $\text{ClO}_4^-$ , or  $\text{SCN}^-$  in order of increasing effect. He concluded that  $\text{Cl}^-$  efflux is the sum of two processes: (a) an exchange for penetrating anion which depends on the composition of the external medium, and (b) a net efflux of  $\text{Cl}^-$  in association with cation. Tosteson (272) reported a similar effect in beef red cells: the rate constant for  $^{35}\text{Cl}$  efflux was 3.1, 1.8, and 0.6  $\text{sec}^{-1}$  in  $\text{Cl-PO}_4$ ,  $\text{Br-PO}_4$ , and  $\text{I-PO}_4$  buffer, respectively.

Anion transfer rates in proportion to naked rather than hydrated ion size were also observed in gustatory cells. The electrical response in the frog tongue was reduced in the order  $\text{SCN}^- > \text{NO}_3^- > \text{I}^- > \text{Br}^- > \text{Cl}^-$  [Kusano & Sato (153)]. Active transport of  $\text{I}^-$  by the rat small bowel was postulated by Acland & Illman (3), but they failed to rule out possible coupling to a cation transport system in accounting for the observed concentration gradients.

*Transport of weak electrolytes.*—Lipid solubility and electrical charge are especially important in determining the rates of passive transit and steady-state concentration gradients of weak electrolytes. Sperber (257) reviewed the secretion of organic anions in bile and urine. Milne *et al.* (188) provided a very good review of the literature on the transport and accumulation of weak acids and weak bases in biological systems. They emphasized that in reactions of the type:



the un-ionized species will cross biological membranes much more quickly than the ionized species and, consequently, weak bases will be trapped in acidic fluids and weak acids in alkaline fluids. Schanker *et al.* (117, 239) discovered that acidic drugs were rapidly absorbed across the small bowel of the rat if the  $\text{pK}_a$  was greater than three, while basic drugs were rapidly absorbed if the  $\text{pK}_a$  was less than eight. Raising the pH of the perfusates from four to eight resulted in decreased absorption of acidic drugs and increased absorption of basic drugs, indicating preferential absorption of the un-ionized form. The rates of absorption and the oil/water partition coefficients correlated sufficiently to support the assumption that the un-ionized forms penetrate the cell across a barrier rich in lipids. Other examples of the role of pH gradients and lipid solubility in the transport of weak electrolytes are: (a) the trapping of the weak base, neutral violet, in the gastric juice in proportion to  $\text{H}^+$  secretion [Bilski & Öbrink (20)], (b) the passage of  $\text{NH}_3$  across the blood-brain barrier and its accumulation in brain [Stabenau *et al.*

(260)], (c) the partition of a wide variety of weak organic electrolytes of pharmacological interest between blood, cerebrospinal fluid, and brain [Rall *et al.* (215); Mayer *et al.* (180)], and (d) the permeability of living membranes to  $\text{CO}_2$  and their impermeability to  $\text{HCO}_3^-$  [Halpern & Binaghi (100)].

In some systems, however, the permeability coefficients may be decisive in determining accumulation rather than pH gradients. Under inhibition by dinitrophenol, lactate accumulated selectively on the serosal side of the intestinal wall according to Pfleger *et al.* (210). Similarly, in the isolated toad bladder, lactate transferred rapidly into serosal solutions, despite the absence of a pH gradient [Leaf (160)]. In some systems pH dependence and carrier transport or permeability barrier factors may operate simultaneously to determine transport rates. The addition of hypoxanthine to human erythrocyte suspensions depressed the rate of uptake of uric acid to 20 per cent of the control value, but decreasing the external pH accelerated uptake [Overgaard-Hansen & Lassen (201)].

Amino acid transport should depend on active and selective mechanisms since protein synthesis requires specific ratios of amino acids for specific structures. Heinz & Walsh (109) observed a proportionate increase in the glycine influx coefficient and almost no change in the efflux coefficient with increasing intracellular glycine concentrations in ascites tumor cells and postulated a common carrier-mediated process for glycine exchange and active transport. The participation of pyridoxal and  $\text{Mn}^{++}$  in amino acid transport was proposed by Pal & Christensen (204) because pyridoxal and a number of amino acids stimulated  $\text{Mn}^{++}$  accumulation in ascites tumor cells.

Transport of amino acids across the mucosa of the small bowel shows a high degree of stereochemical as well as chemical specificity. The L isomers of methionine, histidine, cystine, and cysteine are more rapidly absorbed than the D isomers; uptake of L-methionine but not D-methionine is inhibited by dinitrophenol, and L-methionine inhibited L-histidine transport but L-histidine had little effect on L-methionine absorption [Jervis & Smyth (129, 130); Neil (196); Paine *et al.* (203)].

*Metabolic aspects of ion transport.*—The primary issues under debate are (a) the nature of the transport mechanism in the membrane, and (b) the proximate sources of energy for transport.

Coupling of amino acid and  $\text{K}^+$  transport was proposed by Riggs *et al.* (225) because in ascites tumor cells, glycine accumulation required a normal cell  $\text{K}^+$  concentration and pyridoxal stimulation of tryptophan, and glycine uptake required  $\text{K}^+$  in the medium. The specificity of this relationship, however, has not been established since many metabolic functions are impaired by  $\text{K}^+$  depletion. Wang & Koblick (287) proposed that acetylcholine may be directly involved in ion transport since inhibition of choline acetylase depressed the short-circuit current and transepithelial potential difference in the frog skin. If acetylcholine does participate in the carrier system, cholinesterase should be needed as a regulator. However, cholinesterase defi-

ciency had no effect on unidirectional  $K^+$  fluxes in erythrocytes [Auditore *et al.* (9)].

Carbohydrate metabolic pathways are closely linked with ion transport, very probably by providing high-energy phosphate compounds for the transport mechanism. In the isolated, perfused frog liver, epinephrine induced  $K^+$  loss and net glucose and lactate efflux, while iodoacetate decreased lactate production and stimulated  $K^+$  and glucose loss [Craig (44)]. In general, however,  $K^+$  loss preceded glucose loss, which is evidence of an indirect rather than a direct association of these events. Oxidative mechanisms ultimately are required to maintain transport mechanisms. In newborn lambs, the concentration gradient of  $K^+$  across the red cell declined roughly in proportion to the decline in erythrocyte oxygen consumption and had no consistent relation to lactate production [Wright *et al.* (296)]. Maintenance of oxygen consumption does not, however, assure an adequate supply of energy for transport since dinitrophenol (DNP) treatment impairs almost all such processes. Sorokina (254) found that DNP, as well as iodoacetate and  $NaN_3$ , reduced the resting p.d. of striated muscle fibers in about two hours *in vitro*. Woodbury (295) argued that the effects of DNP on the resting p.d. of frog sartorius could not be explained either by a change in the  $Na^+$  permeability coefficient or by inhibition of the  $Na^+$  extrusion process because addition of DNP to  $K^+$ -free solutions caused a rapid fall in p.d., which was not affected by substitution of sucrose for 80 per cent of the  $NaCl$ , followed by a recovery to near control values. However, salicylates, which also act to uncouple oxidative phosphorylation, also block active transport in red cells and in muscle. Addition of salicylate to human red cell suspensions resulted in  $K^+$  loss and  $Na^+$  gain in a ratio of about five to one [Waltner *et al.* (285)]. Salicylate-treated rat diaphragm segments lost cellular  $K^+$ , but both  $K^+$  influx and  $K^+$  efflux were increased, according to Hicklin (110). This may indicate a metabolically dependent component in the passive permeability properties of the sarcolemma.

The data cited above, as well as many earlier studies, provide some basis for supposing that a high-energy phosphate compound (either ATP or a closely linked species) is the intimate substrate for many if not all transport processes. A final answer is not at hand but arguments pro and con are available in abundance.

(a) Evidence against a high-energy phosphate compound as the energy donor for ion transport. Carey *et al.* (34) showed that active  $Na^+$  extrusion occurred in frog sartorius if preloading of the cell was accomplished by incubation at  $0^\circ C$ . in  $K^+$ -free Ringer's with a  $Na^+$  concentration of 120 mM and recovery was allowed to take place at room temperature in 104 mM  $Na$ -10 mM  $K$ -Ringer's solution. Iodoacetate (2mM), ouabain (10 mM), and cold ( $0^\circ C$ .) fully inhibited active  $Na^+$  transport, CN (2 mM) and anoxia produced 60 per cent inhibition, but DNP (0.027 mM) stimulated  $Na^+$  secretion by about 10 per cent. Estimated ATP content was about 25 per cent



less in DNP-treated muscle after incubation at room temperature than in muscle incubated without DNP. The authors argued that ATP does not supply the energy for  $\text{Na}^+$  transport and that the data fit with the Conway redox pump theory although the redox pump may be backed by oxidative phosphorylation. Their findings, however, do not contradict the ATP hypothesis since they failed to deplete the cell of high-energy phosphate compounds before induction of  $\text{Na}^+$  extrusion. Indeed, the fall in ATP under DNP treatment may be, in part, a consequence of its utilization for  $\text{Na}^+$  transport.

Potassium influx in human erythrocytes, according to Eckel (69), is reduced only to 25 per cent of normal by 0.005 *M* NaF. In a later study, however, he found that in 0.025 *M* NaF the  $\text{K}^+$  influx:efflux ratio approached the prediction of passive movement and that the ATP content of the red cell declined more than the ADP content (70). Conceivably, therefore, the component of  $\text{K}^+$  influx coupled to the active transport mechanism may be ATP dependent.

Brady *et al.* (25) noted that injection of ATP (0.05 *M*) into squid axon axoplasm had no effect on the action potential, but neither did intraaxonal injection of acetylcholine. Consequently, the ATP may not have reached the site of transport; alternatively, endogenous ATP may have saturated the mechanism. In single skeletal muscle fibers of the frog, Voronova (281) observed that  $6 \times 10^{-4}$  *M* DNP had no effect on accumulation of  $^{42}\text{K}$  or  $^{24}\text{Na}$  during 10-minute periods of rest, excitation, or recovery. Endogenous reserve of high-energy phosphate compounds, however, may have been adequate for the energy required during these brief periods of study.

In frog skeletal muscle and nerve, substitution of hydrazinium ion for sodium ion did not abolish normal electrical activity despite the almost complete disappearance of phosphocreatine and the depression of ATP-turnover estimated by  $^{32}\text{P}$  exchange [Abood & Koketsu (2)]. Since exchange of  $^{32}\text{P}$  from labeled ATP may occur without ATP degradation and inasmuch as other phosphate donors may supply high-energy phosphate to a small but rapidly exchanging fraction of ATP, these data do not exclude ATP or related compounds from a primary role in active transport processes. An example of an effect on  $^{32}\text{P}$ -labeled ATP exchange without an obvious effect on the rates of ATP degradation or synthesis is given by Harris & Prankerd (102), who found that phlorizin depressed the specific activity of  $^{32}\text{P}$ -labeled ATP without changing either ATP, sodium or potassium content of red cells. In patients with diabetic acidosis, Jones (131) noted depletion of red cell  $\text{K}^+$  and 2,3-diphosphoglycerate despite normal  $\text{Na}^+$  and ATP concentrations. A block in the use of ATP for transport, however, could account for the failure to maintain  $\text{Na}:\text{K}$  gradients in the presence of a normal supply of ATP.

(b) Evidence in favor of a high-energy phosphate compound as the energy donor for ion transport. High-energy phosphate ester content correlated



with Na:K gradients in human red cells separated into young and old fractions [Bernstein (17)]. Moreover, adenosine maintained the labile phosphate ester and low sodium contents of swine red cells during storage [Kirschner & Harding (143)].

In Na<sup>+</sup>-loaded frog sartorii treated with 2 mM NaCN, the ratio of net Na<sup>+</sup> extrusion to oxygen consumption exceeded the redox pump (unmodified by an ATP booster) prediction of a 4:1 molar ratio of Na/O<sub>2</sub> [Frazier & Keynes (83)]. From separate experiments on oxygen consumption and active sodium transport across the isolated toad bladder, Leaf *et al.* (161) calculated an average Na<sup>+</sup>/O<sub>2</sub> ratio of 16.5. Exposure of chicken nucleated red cells to KCN at 4°C. did not inhibit K<sup>+</sup> retention or promote Na<sup>+</sup> gain, while iodoacetate definitely impaired the maintenance of the electrolyte gradients [Banaschak (11)]. The rate of fall of cellular ATP content, however, was greater with KCN than with iodoacetate. Nevertheless, in these systems the link between oxygen consumption and sodium transport appears to be indirect and probably is mediated via the oxidative synthesis of high-energy phosphate esters.

Despite their rejection of the membrane transport theory, Briner *et al.* (28) did find that toad sartorius incubated in  $2 \times 10^{-3}$  M iodoacetate showed no change in Na<sup>+</sup>, K<sup>+</sup>, or ATP content when quiescent, and a simultaneous fall in K<sup>+</sup>, gain in Na<sup>+</sup>, and marked lowering of ATP and creatine phosphate content when spontaneous twitching supervened. Similar correlations were seen with other metabolic inhibitors (DNP, methylene blue, and NaN<sub>3</sub>). Repetitive stimulation of C fibers of the rabbit vagus at 50 sec.<sup>-1</sup> for 15 sec. produced a decrease of about 17 per cent in their ATP and creatine phosphate content [Greengard & Straub (95)].

Electrical activity was inhibited and the resting potential declined in DNP-treated single cells of the sinus node, right auricle, and auriculoventricular node; these changes were partially reversed by addition of ATP to the medium [de Mello (58)]. Also, ATP added to the medium partially restored the electrical activity of frog ventricles paralyzed by elevation of K<sup>+</sup> to 12.8 mM [Kotowski *et al.* (147)]. Dudel & Trautwein (66), however, were unable to confirm the earlier report of Fleckenstein that externally applied ATP hyperpolarized the frog myocardium. Furthermore, ATP, because of its considerable capacity for binding divalent cations, could affect transport processes without being used as an energy donor by the system.

Whittam & Breuer (288) noted an approximate correlation between easily hydrolyzable phosphate esters and maintenance of sodium and potassium gradients in slices of guinea pig seminal vesicle mucosa after DNP, iodoacetate, or anaerobiosis. The contralateral renal cortex, after unilateral nephrectomy, showed parallel changes in electrolyte content and readily hydrolyzable phosphate ester content [Breuer (26)]. Moreover, Spater *et al.* (255) reported that the ascending loops of Henle and the infolded cell membranes of the proximal tubules of the mammalian kidney are rich in adenosinetriphosphatase activity.

A scheme for the active transport of hydrophilic species by a phosphate transfer mechanism was proposed by Hokin & Hokin (118). In essence, the carrier, phosphatidic acid, releases the passenger by the action of phosphatidic acid phosphatase and then is reformed from ATP. To support this concept they cite the stimulating effect of acetylcholine on the formation of phosphatidic acid from ATP.

*Hormonal regulation of ion transport.*—In general, the molecular mechanisms and the role of hormonal control in ion transport remain enigmatic and probably will continue so until *in vitro* systems are developed which show clear-cut hormonal responses. The findings of Williams & Angerer (292) that frog skin potential difference is depressed by adrenalectomy and increased by adrenal extract may be promising. Contradictory results were obtained in red cells: Friedman & Friedman (84) said that aldosterone blocked  $\text{Na}^+$  extrusion from human red cells, while Kunz & Sulser (152) reported that after adrenalectomy, rat red cells tend to accumulate more  $\text{Na}^+$  on cold storage. Although Briggs & Holland (27) found that deoxycorticosterone acetate depressed  $\text{K}^+$  efflux in isolated rabbit atria and in higher concentrations reduced  $\text{K}^+$  influx, aldosterone, the primary mammalian salt-regulating hormone, had no effect on  $\text{K}^+$  fluxes. Furthermore, injection of aldosterone in adrenalectomized-nephrectomized rats had no effect on the estimated extracellular content of  $\text{Na}^+$  or  $\text{K}^+$  [Friedman *et al.* (85)].

In contrast to the more obscure role of adrenal cortical hormones in transport processes, insulin appears to regulate the permeability characteristics of a variety of cell membranes. Despite the absence of glucose from the medium, insulin increased the resting potential difference of rat skeletal muscle prior to any change in  $\text{K}^+$  gradient [Zierler (298, 299)]. In the isolated rat diaphragm, insulin also produced a three- to fivefold increase in the rate of accumulation of  $\alpha$ -aminoisobutyrate, a metabolically inactive amino acid [Kipnis & Noall (140)]. D'Amico & Foá (51), however, observed no effect of insulin on the  $\text{K}^+$  uptake by erythrocytes taken from a patient in diabetic coma, nor did insulin reverse ouabain inhibition of  $\text{K}^+$  uptake.

Although transport mechanisms in uterine muscle and endometrium may respond specifically to estrogens and progesterones, the evidence for this is fragmentary. Estrogen treatment of ovariectomized rats induced rhythmical contractions and raised the uterine muscle fiber resting potential difference from a control of 35 mv. to a value of 58 mv.; progesterone-dominated fibers had even higher membrane potentials (64 mv.) [Marshall (178)]. Endometria of progesterone-treated rabbits were high in  $\text{K}^+$  and low in  $\text{Na}^+$  compared to estrogen-dominated endometria [Bitman *et al.* (21)]. As emphasized by Spaziani & Szego (256), these changes may simply be a result of estrogen-induced uterine hyperemia.

*Acid-base equilibrium.*—The regulation of  $\text{H}^+$  concentrations (or more precisely,  $\text{H}_3\text{O}^+$  concentrations) is intimately associated with a great variety of metabolic and transport processes. However, progress in this branch of physiology has been delayed in particular by the technical difficulty of

measuring intracellular pH. Caldwell (33) described a microelectrode method for measuring intracellular pH in large fibers. Immersion of crab muscle and squid giant axon, at constant  $p\text{CO}_2$ , in saline solutions or sea water produced little change in pH; saturation with  $\text{CO}_2$ , however, resulted in a rapid decrease in pH. He deduced that the  $\text{H}_2\text{CO}_3\text{-HCO}_3^-$ -buffer system regulates cellular pH and that  $\text{HCO}_3^-$  permeability is slight compared to  $\text{CO}_2$ .

Gradients of  $\text{H}^+$  have a striking effect on monovalent cation distribution and transport, probably because the small size and high conductivity of  $\text{H}_3\text{O}^+$  enable it to act as a universal competitor for cation transport mechanisms. In contrast to HCl, the addition of organic acids to the isolated rat diaphragm did not promote loss of cellular  $\text{K}^+$  [Rogers & Wachenfeld (228)]. Tobin (271) confirmed the differential effects of mineral and organic acids on cellular  $\text{K}^+$  in bilaterally nephrectomized cats. Leibman & Edelman (165) estimated  $\text{K}^+_{\text{o}}/\text{K}^+_{\text{i}}$  gradients from measurements of plasma  $\text{K}^+$  and the exchangeable potassium pool (expressed as  $\text{K}_\text{e}$ /dry body weight) and found a high degree of correlation between plasma  $\text{K}^+$  and the product plasma  $\text{H}^+ \cdot \text{K}_\text{e}$ /d.b.w. ( $r=0.7$ ) in patients with metabolic-acid base disturbances, but little correlation ( $r=0.2$ ) in patients with respiratory acid-base disturbances. They interpreted these findings as evidence of a modified Donnan distribution for  $\text{H}^+$  and  $\text{K}^+$  across cell membranes. Metabolic acid-base disturbances were presumed to induce changes in  $\text{H}^+$  gradients and, as a consequence, corresponding shifts in  $\text{K}^+$ . In anesthetized dogs, regulation of  $p\text{CO}_2$  to maintain a constant arterial pH during administration of HCl or  $\text{NaHCO}_3$  prevented the usual reciprocal changes in plasma  $\text{K}^+$  [Simmons & Avedon (249)].

Eckel *et al.* (71) were unable to confirm earlier reports by others of an intracellular acidosis when cell  $\text{K}^+$  is depleted. The calculated pH of muscle homogenates based on  $\text{CO}_2$  partition and direct measurement of homogenate pH showed no differences in normal and  $\text{K}^+$ -deficient muscle. Furthermore, it now appears that metabolic alkalosis occurs as a consequence of  $\text{K}^+$  depletion only in the presence of  $\text{Na}^+$  retention. Dietary restriction of  $\text{Na}^+$  and  $\text{K}^+$  in dogs did not evoke metabolic alkalosis until either supplementary  $\text{Na}^+$  or corticosteroids were given [Muntwyler *et al.* (193); Grollman & Gamble (96)]. Moreover, cumulative  $\text{K}^+$  losses as high as 6.4 m.eq. per kg. of body weight were not attended by alkalosis in man until deoxycorticosterone was administered [Squires & Huth (259); Huth *et al.* (121)].

#### TRANSPORT OF SUGARS

Most of the recent work has been concerned with the mechanism of hexose transport across cell membranes, the energy requirements for this process, the role of insulin, and the mechanism of action of inhibitors, notably phlorizin and phloretin. Park *et al.* (205) reviewed the recent literature on this topic, with primary emphasis on restricted diffusion and carrier-mediated transport.

*Sugar transport in erythrocytes.*—LeFevre & Marshall (163) discovered that in human red cells the dissociation constants for the presumed carrier-sugar complexes varied inversely with relative stability in the "C-1" chair conformation, and postulated that the C-1 conformation provides steric specificity for combination with the carrier.

Estrogens, which resemble phloretin in the spacing between terminal phenolic-OH groups, also inhibit the influx of hexoses in human red cells, presumably by combining with regularly spaced membrane groups capable of reversible binding with phenolic-OH [LeFevre (162)]. The number of sugar-transporting sites, however, probably constitutes only a small fraction of the inhibitor binding sites since the amounts of bound phloretin or estrogen exceeded by far the amounts required for maximal inhibition of glucose transport [LeFevre & Marshall (164)]. Bowyer & Widdas (23) proposed an enzyme carrier containing a glucose acceptor and a phosphate donor site at each interface of the cell membrane as a model for glucose transport. The glucose moiety is released into the cell after the formation of free glucose phosphate by enzymic hydrolysis and the subsequent transfer of the phosphate to the acceptor at the inner boundary of the membrane. Although this mechanism is consistent with recent results, it has not yet been subjected to a critical experimental test.

Glucose utilization does not regulate glucose transfer in red cells inasmuch as complete inhibition of utilization by sodium fluoride or iodoacetate only halves glucose uptake and as phlorizin does not impair utilization [Laris (157)]. The rate of transit, however, depends strongly on the chemical structure of the hexose. In rabbit red cells, the order was glucose > alkylglucose > deoxyglucose, according to Hillman *et al.* (111). Furthermore, increasing the chain length of the aliphatic substituent greatly accelerated the transfer rate. All the analogues probably gain entrance via the same membrane sites since they all inhibited glucose uptake competitively.

*Sugar transport in muscle.*—The ability of insulin to augment cellular permeability to hexoses is partially specific. According to Landau *et al.* (154), insulin increases the volume of distribution of 2-deoxyglucose, 3-deoxyglucose, and 2-deoxygalactose but not of 6-O-methylglucose, indicating that a C-2- or C-3-OH is not required for an insulin response. Insulin may act by binding to membrane carrier sites since phlorizin reversed the insulin expansion of the galactose volume of distribution in eviscerated rats [Keller & Lotspeich (136)]. Randle and co-workers (191, 216, 217) argued that insulin promotes glucose transport in muscle by preventing access to the membrane of an inhibitor generated by oxidative phosphorylation, because in the rat heart or diaphragm, insulin potentiated the increased glucose uptake induced by anaerobiosis or by a variety of metabolic inhibitors.

Hexose transport across the membrane and phosphorylation appear to be linked but separate events. Kipnis & Cori (139) stated that the capacity of the isolated rat diaphragm to phosphorylate deoxyglucose exceeded the

rate of penetration even in the presence of insulin but that the accumulation of 2-deoxyglucose- $\text{PO}_4$  inhibited uptake of 2-deoxyglucose and glucose but not of 2-deoxygalactose or D-xylose. The alkali-metal ions also affect the glucose transport mechanism since according to Bhattacharya (19), glucose uptake in the isolated rat diaphragm varied in the order  $\text{Li}^+ > \text{Na}^+ > \text{K}^+ = \text{Rb}^+ > \text{Cs}^+$  in isomolar solutions.

*Sugar transport across the small bowel.*—The everted sac technique, described in detail by Crane & Wilson (46), has been used widely to study transport of sugars across the small intestine *in vitro*. The specificity of active mucosal to serosal transport was tested with 28 sugars by Wilson & Crane (293): a D-pyranose structure, a methyl or substituted methyl group at C-5, and a C-2-OH group were required for active transport. Neither removal of the C-2-OH nor conversion of the hexoses to trioses, however, appears to be involved in transport through the intestinal mucosa [Crane & Crane (45); Landau & Wilson (155)].

In contrast to their action in skeletal and cardiac muscle, compounds that uncouple oxidation and phosphorylation suppress hexose transport through the intestinal mucosa: DNP, salicylates, and triethyl tin sulfate abolished active glucose transfer in this system [Smith (253); Parsons (206); Rummel *et al.* (235)]. It is interesting that Rummel *et al.* (235) observed reactivation of DNP-inhibited glucose transport by  $\text{NaN}_3$ , iodoacetate, and NaF, agents which suppress glycolysis, suggesting that ATP is not required. Although mucosal creatine phosphate or ATP content was unchanged, however, the rate of incorporation of  $^{32}\text{P}$  into many organophosphates (fructose-diphosphate, triose phosphate, creatine phosphate, and adenosine, uridine, and guanosine triphosphates) was much faster during active glucose transport than during absorption of glucose-free water [Janke *et al.* (127)]. The role of phosphorylation in intestinal transport of hexoses, therefore, is still *sub judice*. Intravenous injection of cytochrome-c stimulated glucose and arabinose absorption from the small bowel of the rat according to Ponz & Lluch (212).

The ionic composition of the medium governs the rate of glucose transport across the small bowel: potassium stimulates glucose absorption by the guinea pig intestine provided that  $\text{Na}^+$  and  $\text{Mg}^{++}$  are present, whereas  $\text{NH}_4^+$  is inhibitory [Riklis & Quastel (223)]. In addition,  $\text{K}^+$  stimulation of hexose transport is suppressed by DNP, malonate, and phlorizin [Riklis and co-workers (222, 224)]. On the basis of these results and the competition between related sugars for transport, they posited that an enzymatically driven phosphorylation mechanism other than hexokinase controls hexose transport through the intestinal mucosa. However, Jacobi *et al.* (125) observed a higher  $Q_{10}$  for  $\text{PO}_4$  than for glucose transfer across the intestinal wall. Also, DNP and phlorizin quenched active glucose transport but increased  $\text{PO}_4$  absorption, indicating that net transport of these species may be unrelated.

*Sugar transport in other systems.*—The glucose molecule apparently remains intact in transit through the proximal tubule of the kidney since Chinard *et al.* (38), after infusing either glucose-1- $C^{14}$  or glucose-6- $C^{14}$ , recovered 98 per cent of the filtered glucose- $C^{14}$  in renal vein blood without any evidence of randomization.

Burger *et al.* (31) noted competitive inhibition of hexose influx rates between D-galactose, D-arabinose, glucose, and glucosamine in yeast cells. Best (18) argued that glucose transport across the yeast cell membrane is not accomplished by an enzymatic-carrier system because uranyl-ion inhibition of glucose uptake did not significantly alter the enzymic parameters in a Michaelis-Menten kinetic analysis.

#### TRANSPORT IN TISSUES, ORGANS, AND INTACT ANIMALS

*Transport in systems with hydrodynamic flow.*—Analysis of membrane transport phenomena in tubular structures such as the nephron or capillary bed is complicated by the simultaneous hydrodynamic flow at right angles to the transport process. Bergmann & Dikstein (15) derived general equations for the transfer of solutes through the walls of cylindrical tubes when axial flow predominates. The usefulness of these equations, however, will depend on whether all the critical parameters can be measured or controlled in any specific experiment.

One of the primary difficulties in flow system experiments is heterogeneity in perfusion rates when many tubular structures are perfused in parallel. Analysis of the regional tissue  $D_2O$  concentrations during brief perfusions with  $D_2O$ -labeled fluids revealed discrepancies between the observed and theoretical venous outflow  $D_2O$  curves in perfused organs suggestive of heterogeneity in flow/tissue mass ratios [Thompson *et al.* (268)]. As Renkin (220, 221) pointed out, the calculation of cellular exchange from tracer curves during vascular perfusion requires that both local perfusion rates and interstitial fluid isotope concentrations remain reasonably homogeneous. He found that these assumptions were not valid for dog skeletal muscle perfused with blood tagged with  $^{42}K$ .

*Heart.*—The recent literature on this topic is well covered by Hoffman (115) in this volume and by Hajdu & Leonard (99).

The following studies provided additional corroborative evidence for the membrane transport-ion gradient theory: (a) perfusion of  $K^+$ -enriched Ringer's solution in the toad decreased the resting potential difference and broadened the action potential in heart fibers [Trethowie & St. Clair (272a)], (b) spontaneous cessation of contraction in isolated atria occurred at levels of  $K^+$  depletion which would bring the resting potential below the critical level for maintenance of excitability [Goodford (92)], and (c) the speed with which  $Ca^{++}$  and strophanthin exert their inotropic effects on the rat heart speaks for a surface membrane locus of action [Reiter (219)].

*Skeletal muscle.*—Analysis of human skeletal muscle by revised techniques

based on exclusion of  $\text{Cl}^-$  from the fiber water yielded intracellular concentrations of 164 m.eq. per l. for  $\text{K}^+$  and 11 m.eq. per l. for  $\text{Na}^+$  [Litchfield & Gaddie (167)]. These estimates agree with the values derived for intact subjects based on a revised scheme of the organization of body water and electrolytes [Edelman & Leibman (73)].

From chemical analysis and  $^{42}\text{K}$  exchange, Baker *et al.* (10) inferred that depletion of muscle cell  $\text{K}^+$  and excess of  $\text{Na}^+$  accompany the genetic myopathy of hereditary dystrophy in mice. Young *et al.* (283), however, concluded that the low  $\text{K}^+$ /high  $\text{Na}^+$  content of such muscle could result from the marked increase in extracellular solids rather than from abnormalities in cellular  $\text{Na}^+$  and  $\text{K}^+$  concentrations.

Hashish (106) wrote that  $^{42}\text{K}$  exchange in rat diaphragm at  $1^\circ\text{C}$ . is increased markedly by heparin, suggesting that the low-temperature block to  $\text{K}^+$  exchange may be a membrane rather than a cytoplasmic effect. Replacement of  $\text{Na}^+$ -Ringer's with  $\text{NH}_4^+$ -Ringer's increased the frequency of endplate potentials in isolated amphibian sartorii, particularly in the presence of acetylcholine [Furukawa *et al.* (87)]. This raises the possibility that acetylcholine decreases the resistance to ionic transit by expanding the aqueous channels in the endplate membrane since the limiting ionic conductance of  $\text{NH}_4^+$  is considerably greater than  $\text{Na}^+$  conductance. [See Mommaerts & Abbott (189) for additional studies on this topic.]

*Smooth muscle.*—Daniel (52), in reviewing some of the recent data on mammalian smooth muscle, concluded that  $\text{Cl}^-$  distribution is compatible with electrochemical equilibrium and that  $\text{Na}^+$  and  $\text{K}^+$  are actively transported.

Transport processes in smooth muscle are very sensitive to  $\text{Ca}^{++}$ . Addition of  $\text{Ca}^{++}$  to the medium slowed the rate of  $\text{K}^+$  loss and  $\text{Na}^+$  gain in rabbit uterine segments and restored excitability [Daniel & Daniel (53); Coutinho & Csapo (43)].

*Nervous system.*—The reviews in this volume should be consulted for more data on transport in relation to neurophysiological mechanisms.

An interesting divergence in glutamate uptake and its ability to promote  $\text{K}^+$  storage in slices of guinea pig cortex was reported by Takagaki *et al.* (263): the slice to medium ratio of D-glutamate concentration was five times greater than the L-glutamate ratio, but the latter was the more potent accelerator of  $\text{K}^+$  uptake.

Iodide, *p*-aminohippurate, and sucrose penetrate the brain very slowly when injected intravenously but enter the substance of brain slices readily from an incubation medium [Davson & Spaziani (56)]. These authors argued therefore, that the brain-vascular system contains a major diffusion barrier and rejected the concept of an absence of an interstitial fluid volume in brain. Tschirgi & Taylor (274) described a  $\text{H}^+$ -sensitive p.d. between the substance of mammalian brain and jugular vein blood and postulated a source of electromotive force in the vascular blood-brain barrier. A similar



concept of an ion-transporting system at the site of the blood-brain barrier was advanced by Coulter (42) because he obtained a hydrodynamic filtration coefficient in brain in excess of that in skeletal muscle. Neither argument is compelling, however, since the potential difference measurements were not made across a well-defined boundary and the estimate of the filtration coefficient is a measure of the flow of water, rather than solute. An interesting technique for chronic ventricular perfusion was described by Manuilov (177).

*Gastrointestinal tract.*—Janowitz (128) and Lundberg (169) should be consulted for recent literature on this topic.

Davenport & Allen (55) rejected the Rehm hypothesis that gastric  $H^+$  secretion originates in the surface epithelial cells. They denuded mouse stomachs of an estimated 97 per cent of these cells and demonstrated acid secretion which responded normally to carbachol and atropine sulfate. In isolated frog gastric mucosa and intact dog stomach, raising the  $K^+$  concentration on the capillary side depressed the transmucosal p.d. and in the former preparation increased  $H^+$  secretion [Harris *et al.* (104); Chandler *et al.* (37)]. Harris & Edelman (103) proposed that the effects of nutrient  $K^+$  concentration on  $H^+$  secretion and on transmucosal p.d. are independent because raising nutrient  $K^+$  in the presence of either thiocyanate inhibition or histamine stimulation of  $H^+$  secretion depressed transmucosal p.d. Moreover, Heinz & Durbin (108) eliminated active  $Cl^-$  transport by replacing nutrient  $Cl^-$  with  $SO_4^-$  and showed that the reversed short-circuit current corresponded to the rate of  $H^+$  secretion. In effect, they established that the gastric mucosa contains an active  $H^+$  transport mechanism which operates independently of the  $Cl^-$  transport mechanism or spontaneous potential. The Heinz-Durbin postulate was confirmed by Hogben (116), who reported that the elasmobranch stomach secretes  $H^+$  in the absence of a significant potential difference.

*Intact animals.*—A revised scheme of the organization of body water and electrolytes in man consisting of five extracellular subdivisions (plasma, interstitial/lymph fluid, dense connective tissue, cartilage and bone, and transcellular fluid) and the intracellular phase, based on tracer dilution and tissue composition studies, was proposed by Edelman & Leibman (73).

The rule that cellular  $K^+$  cannot increase above the normal level was corroborated by Drescher *et al.* (65): rats fed more than 0.26 m.eq. per gm. $^{-1}$  body wt. $\cdot$ day $^{-1}$  of  $K^+$  died with high plasma  $K^+$  concentrations but no significant change in total carcass  $K^+$  content. Cationic amino acid accumulation in  $K^+$ -depleted muscle apparently is not a simple exchange process, inasmuch as feeding of lysine to rats on a normal  $K^+$  diet did not reduce muscle  $K^+$  despite an increase in lysine content [Eckel *et al.* (72)]. However,  $Rb^+$  displaces  $K^+$  from the intracellular phase in dogs as indicated by a rise in plasma  $K^+$ , increased urinary excretion of  $K^+$  and rapid cellular uptake of the administered  $Rb^+$  [Kunin *et al.* (151)]. In rats, feeding of diets low in



$Mg^{++}$  resulted in proportionate falls in skeletal muscle  $Mg^{++}$  and  $K^+$  content and significant elevation of muscle  $Na^+$  and  $Cl^-$ ; electrolyte contents of brain, liver, and kidney were unchanged [MacIntyre & Davidsson (172)]. Magnesium deficiency, therefore, provides a means for experimental production of selective depletion of muscle  $K^+$ .

Rolf *et al.* (229) asserted that in rats the  $Na^+$  lost after adrenalectomy corresponds to the decline in the  $Na^+$  content of the sucrose space, suggesting that internal shifts are not involved in the development of adrenal insufficiency. Peters (209), on the other hand, reported that administration of aldosterone to adrenalectomized rats raised plasma  $Na^+$  concentration, although no exogenous NaCl was given.

*Miscellaneous.*—Transport processes in the formation of the aqueous humor were well covered in the review by Langham (156). The electrical asymmetry across the capsule of the lens and the chorion indicates that these structures actively transport as yet unidentified ions [Andrée (4); Meschia *et al.* (185)]. If developed to the point of general usefulness, the glass electrodes described by Isard (123) and Friedman *et al.* (86) for measuring the activity of alkali metal ions in solution may prove to be unusually helpful in studies on ion transport.

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# COMPARATIVE PHYSIOLOGY: PHOTOPERIODICITY<sup>1,2,3</sup>

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## INTRODUCTION

The survival of species in an environment with annual fluctuations requires schemes of controls which cause certain critical functions to occur at appropriate seasons (1 to 4). Thus, for example, in the temperate zone most species of birds produce young in spring or summer when food is abundant and days are sufficiently long for feeding them (1). Also, diapause in many temperate-zone species of insects develops in the autumn (5, 6, 7) so that functions with high-energy requirements are suspended during the winter when external sources of energy become unavailable. Although a rapidly growing body of evidence indicates that reasonably accurate biological clocks may be widespread among animals (3, 8 to 11), it appears that most annual physiologic cycles are either controlled, or at least monitored in phase and periodicity, by periodic environmental functions (1, 3, 12). An environmental variable which has such a timing or phasing function has been designated as a *Zeitgeber* by Aschoff (2, 3). The effect of a *Zeitgeber* usually must begin substantially in advance of the period in which the timed function occurs. Thus, if young mammals are to be born in spring, estrus in the female must develop many weeks or several months earlier. Because of its fixed annual periodicity, the change in daily photoperiod frequently is more advantageous than other environmental factors as a timer or *Zeitgeber* in an environment with regular seasons (1). It seems possible that within single orders of animals, and possibly even within families and genera, photoperiodic mechanisms may have evolved more than once independently (1). Recognition of this probability will prevent overzealous generalization.

Among individual species, photoperiodic mechanisms differ extensively in their relative importance. Thus testicular cycles may occur in domestic ducks kept in either continuous dark or in continuous light, showing that the

<sup>1</sup> The survey of the literature was concluded in June 1960. In general this review is based on literature published since 1950, although some older investigations are included. Only a fraction of the available literature is cited because of space limitation. Effort has been directed primarily toward a presentation of the current status of knowledge and principal trends of research.

<sup>2</sup> The preparation of this review was supported in part by a research contract with the Office of Naval Research, NONR-1520(00).

<sup>3</sup> Photoperiodicity is here defined as the control of long-period, usually annual, physiologic cycles by mechanisms which, in some way, are driven or, at least, maintained in phase by the changing length of the natural daily photoperiod. It is used herein as synonymous with photoperiodism (1). I am indebted to Doctor Shunichi Kambara for invaluable assistance in the translation of Japanese papers.



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photoperiodic mechanism functions primarily to maintain fixed periodicity and phase (13). Similarly female ducks and geese, normally cyclic in egg-laying, lay more or less constantly when the daily photoperiod is constant (14). The photoperiodic mechanism in ferrets, too, is certainly not essential for the development of estrus (15) although it does contribute to the precision of the frequency and phasing of the cycle. At the other extreme, photoperiodic controls may approach an absolute status. Within the normal ranges of other environmental variables, this appears to be true in the induction of diapause in some species of insects, such as the Colorado potato beetle *Leptinotarsa decemlineata* (16 to 18) and the western grape-leaf skeletonizer *Harrisina brillians* (19). Also, in the white-crowned sparrow *Zonotrichia leucophrys gambelii*, the photoperiodic mechanism apparently has absolute control over the timing of the vernal complex of events in the reproductive, molting, and migratory cycles (20). Photoperiodically induced diapause in many species of insects requires low temperature for maximum expression (21 to 25).

Although experimental evidence is fragmentary, it appears obvious that there are substantial differences among photoperiodic mechanisms with respect to the actual roles of light and dark. In some birds, at least, the testicular photoperiodic response is apparently dependent only on the length of light period (20, 26 to 29); there is no dark requirement and the dark period has no significance other than that of a period without primary photochemical responses although the light-induced gonadotropic and metabolic effects may continue into the dark period for a brief time (26, 27, 30, 31). However, in many insects in which diapause is induced by short daily photoperiods, there appear to be both light and dark requirements so that diapause does not occur, or is greatly reduced, if either is below a minimum length (32 to 41).

Examples of functions which are induced by "long" daily photoperiods include the vernal complex of reproductive and migratory events in many species of birds [Farner (1, 42, 43)], estrus in ferrets (44 to 46), completion of gametogenesis and reproductive behavior in some fishes (47 to 50), development of female silkworm moths, *Bombyx mori*, which lay diapause eggs (51), and production of parthenogenetic individuals by several species of aphids (24, 52 to 54). Examples of functions induced by "short" days are the elimination of refractoriness to photoperiodic stimulation in some species of birds [Wolfson (55), Emme (56), and Farner (43)], development of winter pelage in mink *Mustela vison* (57 to 59), estrus in sheep (60 to 62) and goats (62, 63), increased sperm production in sheep (64), diapause at various stages in many species of insects [see Lees (5, 24), de Wilde (6), and Emme (4) for résumés], and development of sexual forms of aphids (24, 52 to 54).

There appear to be two different types of fundamental functional roles of the daily photoperiod in the photoperiodic response. In the white crowned sparrow *Z. l. gambelii*, there is a well-defined relationship between the length

of the daily photoperiod per se and the response. A similar relationship exists for many species of insects (5, 23, 24). On the other hand, there is evidence that the change in length of the daily photoperiod may be the basis of the response in some species; examples include the prevention of diapause by increasing the daily photoperiod in the emperor dragonfly *Anax imperator* [Corbet (65)], the induction of diapause in *Nomadacris septemfasciata* by decreasing daily photoperiods [Norris (66)], and possibly the photoperiodic retardation or acceleration of sexual maturity in the domestic fowl by decreasing or increasing daily photoperiods [Morris & Fox (67, 68)].

There are at least superficially striking contrasts in the action spectra of photoperiodic responses. In birds the maximum sensitivity is in the range of about 600 to 750 m $\mu$  [see Farner (43) for résumé]. However, the maximum in the red portion of the spectrum may be more the reflection of greater penetrability (69 to 71). In many insects and in the red-spider mite *Metatetranychus ulmi*, the opposite end of the spectrum is more effective (5, 24, 72).

#### MAMMALS

Although photoperiodicity may be widespread in middle and high latitudes, it has been demonstrated in relatively few species. Involved principally are the control of reproductive functions, and molt and related phenomena. It appears that mammalian photoperiodic mechanisms function primarily as monitors of phase and periodicity. Recent reviews are those of Aschoff (2), Hammond (73), Emme (4, 56), Lobashyev & Savvatyeyev (11), Dutt (74), and Hafez (75).

*Ovarian cycle and estrus.*—There is now evidence that short daily photoperiods induce or accelerate the development of estrus in several species including the sheep (60 to 62, 76 to 79) [see Hafez (60) for a review of earlier literature]; the goat (62, 63); the silver fox *Vulpes fulva* (80 to 82); and the mink *M. vison* [Khronopulo & Drozdova (59); Hammond (57, 58)].

In sheep the daily dark period need not be continuous to obtain the short-day induction of estrus. Hart (61) found 4L, 2D, 4L, 14D, and 4L, 8D, 4L, 8D to be effective, the latter pattern confirmed by Hafez (60); Tsimborovich (83) obtained similar results with 11D, 3L, 3D, 7L and 11D, 3L, 2.5D, 7.5L. Hafez (60) found that a fixed ratio of light to dark (8L, 16D) is sufficient to induce estrus and that a shortening day length is not necessary; this is in agreement with the experiments of Hart (61). Interpretations of photoperiodic experiments in sheep are difficult because of differences in breeds (60, 79); furthermore, apparently no photoperiodic treatment has successfully caused indefinitely long anestrus, emphasizing that photoperiod is not of singular importance in control of the estrous cycle.

Among the species in which estrus has been shown to be induced or accelerated by long days are the ferret (44 to 46, 84, 85) [see Hammond (58) for review of earlier literature]; the raccoon *Procyon lotor* (86); probably the

sable *Martes zibellina* (87); the hare *Lepus timidus* (88); and the horse (89 to 91). Shukyet-Kagan & Emme (92) have shown that estrous periods in white mice are longer and more frequent with long daily photoperiods; this conclusion appears to be consistent with earlier experiments with other species of mice (56, 73).

*Photoperiodic control of estrus in the ferret.*—This is the most extensively investigated photoperiodic phenomenon in mammals. Difficulties in the interpretation of experiments occur because estrus will develop independently of photoperiodic stimulation, the photoperiodic effect being essentially one of acceleration. Thomson (15) has shown that blinded ferrets come into estrus at the normal time and has suggested that photoperiodicity is not involved in the normal timing of the estrous cycle, a view which is perhaps somewhat extreme. The older literature indicating, although not very precisely, that the response is a function of intensity and quality of the light, as well as the duration of the daily photoperiod, has been summarized by Hammond (73). The minimum effective intensity is below 100 lux, and intensities between 100 and 1300 lux are equally effective (93). Maximum response is obtained with daily photoperiods of the order of 14 to 15 hr. (44, 45) suggesting that a dark-period function may be involved in the response (73) or that an alternation of light and dark is essential (15). The long-day effect does not require a continuous long photoperiod (44, 45, 57); indeed a pattern of 2L, 10D, 2L, 10D with a total of only 4 hr. light per day functions as a long day. The receptors apparently are retinal since sectioning of the optic nerves eliminates the response [see Hammond (73) for summary of evidence]. The anterior pituitary is also an essential element in the mechanism (94).

Donovan & Harris (84, 95, 96) found that complete sectioning of the pituitary stalk, and insertion of a wax-paper plate to prevent re-establishment of portal connections with the adenohipophysis, resulted in atrophic ovaries and elimination of the photoperiodic response. Although the path to the hypothalamus and the hypothalamic nuclei involved have not been demonstrated, these experiments indicate that the adenohipophysial portal veins must transport to the adenohipophysis a hormone essential to the response. The experiments and interpretations of Donovan & Harris have been vigorously challenged by Zuckerman & Thomson (46, 97, 339) who argue that the portal vessels are not an essential part of the mechanism. More recently, Donovan & van der Werff ten Bosch (98) found that certain hypothalamic lesions resulted in estrus in winter; they suggest that the lesions may destroy an FSH-inhibiting center which itself would be inhibited normally by long days. Herbert & Zuckerman (99, 100) have argued that such lesions are nonspecific as to location and that estrus is the result of interference with the cerebrum. Abrams *et al.* (101) have shown that cervical sympathectomy delays the onset of photoperiodically induced estrus. This has been confirmed by Donovan & van der Werff ten Bosch (98) who suggest that it may be the result of a decrease in the quantity of light reaching the retina.

*Photoperiodic control of delayed implantation in the Mustelidae.*—The gestation period of several mustelid species can be shortened by long daily photoperiods by reducing the period of delayed implantation [Emme (4)]. This has been demonstrated in several species such as the martin (102), sable (87, 103 to 105), and mink (4, 58, 102).

*Photoperiodic control of spermatogenesis and production of semen.*—Less experimental attention has been given to the photoperiodic control of the male reproductive system. Ortavant (64) has demonstrated that there is an inverse relationship between day length and the quantity of spermatozoa produced by rams, a marked depression being evident with photoperiods greater than 16 hr. Kuznyetsov (80 to 82) also has reported evidence suggesting that decreasing daily photoperiods induces production of semen in the silver fox. In the horse the quantity of semen produced appears to be a direct function of day length (91, 106).

*Molt and related phenomena.*—Photoperiodic control of molt in mammals has been reviewed by Hammond (73), Aschoff (2), and Emme (4, 56). In several species of Mustelidae, it has been demonstrated that short or shortening daily photoperiods cause molt and consequent development of the winter pelage (45, 58, 59, 73, 85, 107). Long or lengthening days apparently cause the molting of the winter pelage in these species although the effect may be indirect and complex (73).

In the silver fox, Bassett (108, 109) has shown that a period of long daily photoperiods (about 18 hr.) in spring followed by a gradual decrease to natural day length in mid-June resulted in an earlier completion of the molt and an earlier development of prime pelts, although the initial date of molt was unchanged. An interpretation of these experiments is difficult although the best hypothesis is perhaps that of an effect of an exaggerated decrease in day length. This is consistent with the experimental acceleration of the development of winter pelage in the same species with artificially shortened days reported by Byelyayev (110 to 112). Similar results have been reported for the arctic fox *Alopex lagopus*, by Markov (113).

The final autumn molt and the development of the white winter pelage of the varying hare *L. americana* require short days, whereas the spring molt and the summer pelage are induced by long daily photoperiods (114); the latter has also been demonstrated in *L. timidus* (88). Long daily photoperiods cause horses (89) and cattle (115) to shed; short daily photoperiods increase wool growth by sheep, an effect which does not occur in hooded animals (116, 117). Little is known concerning the mechanisms involved except that in some cases it has been demonstrated that the eyes and the adenohypophysis are essential elements (73, 114, 116).

#### BIRDS

Photoperiodicity is a widespread phenomenon among birds, particularly in the temperate zones, and is perhaps most important among migratory species (1). Functions known to be directly under photoperiodical control

include reproduction and associated functions [Aschoff (2), Farner (1, 43), Galgano & Mazzi (118), Emme (4), Wolfson (55), Benoit & Assenmacher (119)], vernal migration and the accompanying metabolic changes [Farner (42), Wolfson (55)], and prenuptial molt [Assenmacher (12)]. In at least some species, postnuptial or annual molt, autumnal fattening and migration, and refractory state are photoperiodically induced, although indirectly. Extensive attention has been given to the relation between day length and rate of egg production in the domestic fowl and some attention to the rate of growth and attainment of sexual maturity in several domestic species as a function of day length; however, these functions probably cannot be designated as photoperiodic in the strict sense.

*Testicular function and development.*—In a recent summary (43), acceptable evidence was found for photoperiodic control of the testicular cycle in at least 27 temperate-zone species among 12 families of birds. To this should be added now the brambling *Fringilla montifringilla* (120) and the first case of a transequatorial migrant, the bobolink *Dolichonyx oryzivorus* (121). Photoperiodic testicular development in birds is a "long-day response" with the minimum detectable effect occurring generally at daily photoperiods of the order of 8 to 10 hr.; the rate of testicular growth is then some positive function of the photoperiod for all values that exceed this (1, 20, 43, 122). In the case of the white-crowned sparrow *Z. l. gambelii*, the functional relationship between rate of response and daily photoperiod is sigmoid with the maximum slope coming in between 9- and 17-hr. photoperiods (122).

As noted above, the function of the photoperiodic mechanism varies from apparently complete essentiality in *Z. l. gambelii* (20, 43) to mere monitoring of the phase and frequency of a periodic function which can operate independently, as in the case of the domestic duck (13, 123, 124), and apparently also in the house sparrow *Passer domesticus* (125) and short-tailed shearwater *Puffinus tenuirostris* (126). Miller (127) has shown that in nonmigratory *Z. l. nuttalli* held from mid-winter on 10-hr. daily photoperiods, there was some testicular development by early May, several months after the normal period of development, and that similarly treated, the golden-crowned sparrow *Z. atricapilla* showed a slight testicular development in July. Schildmacher (128) has observed a similar phenomenon in the greenfinch *Chloris chloris* held on 8-hr. photoperiods. Although such changes have been interpreted (127) as an innate testicular cycle, normally phased only photoperiodically, it seems equally probable that it is a normal photoperiodic mechanism operating on near minimum daily photoperiods (43).

The action spectrum has its maximum generally in the wavelength band of about 600 to 750 m $\mu$  [Benoit (129), Farner (43), Benoit & Assenmacher (119)]; and therefore, at least in ducks, it is further toward the red than that of the pupillary reflex (129, 130). The rate of the photoperiodic testicular response is a positive function of light intensity up to an intensity of a relatively low order beyond which the rate remains constant. In the bob-white



*Colinus virginianus*, the maximum rate appears to be attained at about 1 lux with incandescent lamps (131). In *Z. l. gambelii*, the maximum rate is attained at about 30 lux also with incandescent lamps (20). Some effect, at least, can be obtained at intensities as low as 0.1 lux (20, 131). Although it has been suggested that the dark period has some critical role other than the absence of light, as in the photoperiodic responses of some plants (132, 133), such explanations are unnecessarily complex (26, 27); furthermore, experiments with stimulatory photoperiods followed by relatively long dark periods show clearly that only the photoperiod per se is involved in the response in the slate-colored junco *Junco hyemalis* (28), and in *Z. l. gambelii* (20). It has been demonstrated in several species [Farner (1, 43)] that the photoperiodic testicular response does not require continuous light and that effectiveness of a given quantity of light may be increased by giving it in properly spaced short photoperiods (1, 30, 31, 43, 132, 133). Photoperiodic stimulation of males generally produces full testicular development, including production of spermatozoa, and consequent sexual behavior, although the duration of the fully developed state may be shorter than in natural conditions [see, e.g., Vaugien (134, 135)].

*Ovarian function and development.*—Considerably less attention has been given to the photoperiodic control of the ovarian cycle, probably because of its more complex interaction with environmental and behavioral factors. It appears that in most, if not all, passerine species with photoperiodic control of the testicular cycle, an essential initial phase of ovarian development is photoperiodically controlled and that complete development and oviposition usually require additional environmental or behavioral stimuli such as the presence of a photoactivated male, nesting material, and nesting site (136 to 138). Observations on galliform species, including those of Kirkpatrick (131, 139) on *C. virginianus*, suggest that the requirements in addition to photoperiodic stimulation are less complex than in passeriform species; anseriform species appear to be similar in this respect (14). Increased egg production in the domestic pigeon has been obtained by changing from short natural day lengths to 12-hr. daily photoperiods (140).

*The mechanism of the photoperiodic testicular response.*—Nearly three decades of investigations by Benoit, his students, and colleagues have made this response in the domestic duck the most thoroughly understood photoperiodic response system in animals [for integrated reviews, see Benoit (129, 141); Benoit & Assenmacher (119, 142 to 145); Assenmacher (146, 147); Assenmacher & Benoit (148)]. The receptors appear to be both retinal and encephalic (*i.e.*, hypothalamic and rhinencephalic) although neither has been identified morphologically. The action spectrum of the former has a maximum in the orange-red which is distinctly different from the photopic action spectrum; the encephalic receptors are sensitive to almost the entire visual spectrum (149 to 152), although in the intact animals only orange-red light penetrates sufficiently to be of importance (153 to 156). Further essential



elements are neurosecretory cells in the supraoptico-paraventricular nuclear complex of the hypothalamus and their neurosecretion-bearing axons which extend to the glandular layer of the median eminence of the hypothalamus. Lesions in the supraoptico-paraventricular nuclei (147, 157, 158) cause gonadal atrophy and disappearance of neurosecretory material from the "special zone" of the median eminence. Similar results, including failure of the photoperiodic testicular response, are obtained with complete lesions of the hypothalamic neurosecretory tracts to the median eminence (143). The adeno-hypophysial portal system conducts blood from the median eminence to the adeno-hypophysis and constitutes its principal blood supply (147). That it transports a humor essential for gonadotropic function of the adeno-hypophysis is evident from the gonadal atrophy and failure of the photoperiodic testicular response when the portal veins are completely sectioned with no subsequent regeneration (143, 159) and in otherwise successful transplants of the adeno-hypophysis to other parts of the body (147, 148). That the adeno-hypophysis is an essential element in the mechanism is evident further from the failure of the response in hypophysectomized ducks [see Benoit & Assenmacher (119) for résumé of earlier experiments]. The failure of aldehyde-fuchsin or chrome-hematoxylin positive neurosecretory granules to reach the "special zone" of the median eminence, regardless of the experimental manipulation, is accompanied by a failure in the gonadotropic function of the adeno-hypophysis (147).

In *Z. l. gambelii*, Oksche *et al.* (160) demonstrated that the photoperiodic testicular response is accompanied by a decrease in aldehyde-fuchsin stainable neurosecretory material in the cells of the supraoptic nucleus and in the median eminence, the depletion being most noticeable during the latter part of the daily photoperiod; in refractory birds there is a dense accumulation of neurosecretory material in the cells and in the median eminence. Kobayashi & Farner (161) have demonstrated increased acid-phosphatase activity in the supraoptic region and in the median eminence-portal layer during the photoperiodic testicular response.

*The refractory period.*—In passerine species there is a period following a gonadal cycle in which long daily photoperiods fail to cause development of the gonads (1, 29, 43, 55). A similar phenomenon occurs also in the first autumn in most species, the house sparrow *P. domesticus* possibly being an exception (162). Under natural conditions this refractory period, the "preparatory phase" of Wolfson (28, 55), terminates sometime between September and mid-November (43). Although it may be partly coincident with the period of testicular reorganization described by Marshall (163, 164), the refractory period is not the result of refractoriness of the testes as indicated by responses to exogenous gonadotropins (165 to 171). This fact, together with the higher gonadotropin content of the adeno-hypophysis during testicular regression (172), the lack of compensatory testicular growth in hemicastrates during the refractory period (173), and the marked accumulation of neurosecretory material in the hypothalamic neurosecretory cells and in

the median eminence in refractory *Z. l. gambelii* (160), indicates that refractoriness is probably at the hypothalamo-hypophyseal level. The duration of the refractory period is an inverse function of day length (29, 43, 55, 174) so that the shorter natural photoperiods of late autumn, in a sense, constitute a short-day timer which prepares the bird for the vernal photoperiodic responses (1, 175).

A refractory period has been demonstrated in domestic ducks, as well as in passerine species, although it appears to be of shorter duration (173). Among galliform species, it appears that the bobwhite *C. virginianus* does not develop a refractory period similar to that of the photoperiodic passerine species (139, 176). Domestic fowl subjected to long daily photoperiods eventually cease or drastically reduce egg production and molt (177); whether or not this change is homologous with the refractory state of passerine species is not known. The etiology of refractoriness is unknown. Wolfson (175) has concluded that it is somehow caused by long daily photoperiods, a conclusion which is consistent with the suggestion of Threadgold (125) that the photoperiodic response includes both stimulatory and inhibitory processes, the latter being much slower but eventually causing testicular involution and refractoriness. The gonadal feedback hypotheses appear to be inadequate (43), and we [Laws & Farner (178)] have been unable to confirm the bases for a prolactin hypothesis (179, 180). In migratory passerine species, refractoriness also applies to the metabolic responses resulting in fat deposition (42, 181 to 183). In *Z. l. gambelii*, at least, the period of metabolic refractoriness appears to be longer than the period of gonadal refractoriness (182).

*Photoperiodic effects on reproductive functions of the domestic fowl.*—It is a traditional and historic practice among poultrymen to increase egg production of the domestic fowl by artificially increasing day length in winter. Because of the tropical origin of the species and because egg production will continue in total darkness (184), although at a reduced rate, there is the question, perhaps only semantic, as to whether this is a true photoperiodic timing mechanism or simply a light requirement. The latter seems possible as maximum egg production is obtained with daily photoperiods of 12 to 16 hr. (177, 185 to 189). Maximum production can be obtained with considerably less total light per day if it is administered in several or numerous short periods (185, 189 to 193). Less attention has been given to photoperiodic effects on testicular function in the domestic fowl, although it appears that daily photoperiods somewhat in excess of 12 hr. increase production of semen (194) while continuous light (194 to 197) and continuous dark (196) are somewhat inhibitory. There are somewhat similar photoperiodic effects on reproductive functions of domestic guinea fowl (198, 199) and domestic turkey (200 to 202). The photoperiodic effects on the reproductive functions of the domestic galliform species, although somewhat less essential as timers, may be similar to those of the wild galliform species, *C. virginianus* (131, 132, 139, 203, 204).

*Photoperiodic effects on the rate of development of sexual maturity.*—Ac-

celeration of sexual maturity with long daily photoperiods has been reported for the domestic fowl by Karapyetyan (205), Mueller *et al.* (206), Carson *et al.* (207) and others; for domestic ducks by Mori *et al.* (208) and Novikov (14); and for domestic geese by Novikov (14). In the domestic fowl, this acceleration may be more the function of change in photoperiod than a function of the length of the photoperiod per se (67, 68, 209, 210); Mori *et al.* (208) have reached a similar conclusion for domestic ducks. Lobashyev (211) has reported a more rapid sexual development in chickens with a daily pattern of 4D, 8L, 4D, 8L than with 8D, 16L. Novikov *et al.* (212) used the same pattern on Pekin ducks but found no difference in rate of sexual development.

*Molt.*—The physiology of molt, including its photoperiodic aspects, has been reviewed thoroughly by Assenmacher (12). It is possible that in the control of molt there is no general mechanism or battery of mechanisms. Although little attention has been given to the prenuptial molt, it is obviously induced quite directly by long daily photoperiods in the white-throated sparrow *Z. albicollis* (213), *Z. l. gambelii* (20, 214), willow ptarmigan *Lagopus lagopus* (88, 215), and capercaillie *Tetrao urogallus* (12). It has been demonstrated in many species that a molt resembling the postnuptial or annual molt can be induced by feeding desiccated thyroid tissue or by treatment with thyroid hormone or thyroxine, and further, that thyroidectomy or treatment with antithyroid drugs has a delaying, retarding, or inhibiting effect on the annual molt [Assenmacher (12)]. There is some experimental evidence that an increased daily photoperiod induces increased thyroid activity in the domestic duck (216 to 218) and domestic fowl (219). Other similar cases are reviewed by Assenmacher (12). Wilson & Farner (220) were unable to demonstrate histologically a photoperiodic control of either thyroid activity or molt, and a review of the pertinent literature on natural thyroid cycles in passerine species gives no basis for general correlations between length of daily photoperiod and thyroid activity or molt. Recent experimental evidence for the domestic fowl (221, 222) also indicates that the molt does not occur at a time of accelerated thyroid activity. Consequently, the hypothesis of a relatively simple unifactoral relation between the level of thyroid activity and molt cannot be sustained. Among the photoperiodically controlled birds, the annual molt appears to be possible only after a period of long days, during which gonadal development occurs (20, 135, 223 to 226); in these species there is doubtless a mechanism by which there is a "delayed" induction of annual molt by long photoperiods in spring. It is tempting to suggest that in such species molt is induced by an increased ratio of thyroid hormone to sex hormones but this is inconsistent with experiments with castrates (12, 220, 224). Hence, it appears quite possible that this "delayed" photoperiodic induction by long days in spring may well operate through some other mechanism than one involving changes in sex-hormone release incident to the recrudescence and regression of the gonads. Since progesterone has been demonstrated to induce molt (202, 227 to 232), attention should

be directed toward its possible role in the induction of molt at the end of a photoperiodically induced breeding season (12). In most photoperiodically controlled species, the period of the annual molt, like the period of gonadal activity, can be advanced artificially by increasing daily the photoperiod (12). Also, in most of these species, once the bird is in the annual molt, or in the physiologic condition to molt, the annual molt can be advanced or accelerated by decreased daily photoperiods (177, 202, 215, 223, 226, 233 to 236) and retarded by long photoperiods (20, 177, 188, 225, 226, 237).

*Migration.*—There is now sufficient evidence that the complex of physiologic changes which provides the energy for vernal migration and, furthermore, the direct release of migratory behavior are induced by long daily photoperiods in at least several temperate-zone passerine species. The extensive literature has been reviewed by Farner (42, 238), Dorst (239, 240), and Wolfson (55). Natural or experimentally induced migratory behavior, whether detected by experimental releases or by the development of *Zugunruhe* (a distinctive pattern of nocturnal activity in caged subjects), is preceded by a conspicuous deposition of fat in subcutaneous and visceral depots [for a review of the early literature, see Farner (42); for illustrative examples, see Farner (238), King & Farner (241), Wolfson (55, 242, 243), Merkel (244, 245), Wallgren (246, 247), Rautenberg (248), de Bont (249), Odum & Perkinson (341), and Lofts & Marshall (120)]. Actually the entire migratory period is characterized by the ability to restore quickly these depots of fat (42, 238, 246). In at least eight migratory temperate-zone passerine species, it has been demonstrated experimentally that increased daily photoperiods cause and maintain fat deposition (120, 223, 250 to 254). In at least three of these species the major factor in this fattening is a temporary photoperiodically induced hyperphagia (252 to 254). Accompanying this hyperphagia in *Z. l. gambelii* are a reduction of glycogen storage to an inconspicuous minimum, a marked increase in fat content of skeletal muscle and liver, and a marked increase in fat deposition in subcutaneous and visceral depots; these changes are less conspicuous in a more restricted migrant, *J. oreganus*, and absent in the nonmigratory *P. domesticus* (255). Significant experimental investigations on the cause of autumnal fattening and migration are lacking. The best hypothesis is that they are the result of a series of "hour-glass" timers set off by long daily photoperiods in spring.

The timing mechanisms in species which migrate into equatorial regions or across the equator, although possibly containing photoperiodic elements, must be different from those of the temperate-zone migrants (29, 121, 256 to 258).

#### LOWER VERTEBRATES

Relatively little attention has been given to photoperiodicity among the lower vertebrates (1, 118). The photoperiodic control mechanisms appear invariably to involve interactions with environmental temperature.

## FISH

Photoperiodicity in fish has been reviewed recently by Harrington (49). Perhaps the most thoroughly investigated species is the three-spined stickleback *Gasterosteus aculeatus*, which was first shown to be photoperiodic by van den Eeckhoudt (259) and Kazanskii (260, 261). Baggerman (47, 262) has shown experimentally that long daily photoperiods and high temperatures (of the order of 20°C.) cause completion of gametogenesis and, consequently, nest building and oviposition. Simultaneously there develops a preference for fresh water, which causes the pre-breeding migration from salt to fresh water. It appears that the preference for fresh water is caused by increased secretion of thyroid hormone because of stimulation of the adeno-hypophysis-thyroid system by higher temperature and longer daily photoperiods. She (262) suggests that migration back to salt water comes with a less active adeno-hypophysis-thyroid system under conditions of shorter daily photoperiods.

Baggerman (262) has also obtained experimental evidence which suggests that the seaward migration of the coho salmon *Oncorhynchus kisutch* is induced by long daily photoperiods. Northcote (263) has demonstrated that long daily photoperiods (16 hr.) at low temperature (15°C.) cause a negative response to water current in the rainbow trout *Salmo gairdneri*, a response which may be an important component of migratory behavior.

In the goldfish *Carassius auratus*, long daily photoperiods (16 hr.) increase thermal resistance, increase rate of development of ovaries, and suppress thyroid activity slightly (264, 265). Among the cyprinid fishes, Harrington (48, 49, 266) has found that reproduction in the bridled shiner *Notropis bifrenatus* is in part photoperiodically controlled with a postspawning refractory period from mid-July to mid-November; thereafter treatment with long daily photoperiods (17 hr.) causes completion of gametogenesis, prespawning behavior, and spawning. He (49) has demonstrated also a possible photoperiodic component in the control of the ovarian cycle of a low-latitude cyprinodont species, the marsh killifish *Fundulus confluentus*. Long daily photoperiods appear to reinforce the effect of high temperature in retarding oogenesis, but the natural significance of this effect is not yet clear. It appears that the extrinsic control of a protracted period of ovarian activity involves an interaction of temperature and photoperiod; probably the former is more important.

Harrington (267) also demonstrated photoperiodic gonadal control in a centrarchid species, the banded sunfish *Enneacanthus obesus*. In this species, spermatogenesis occurs naturally in the fall but without maturation and without breeding behavior; oogenesis begins naturally in the fall but normally is not completed until spring. Experimentally, in the fall long daily photoperiods (15 hr.) and high temperatures (21.7°C.) caused recrudescence of spermatogenesis, sexual behavior, production of mature ova, and spawning by both sexes.

## AMPHIBIANS AND REPTILES

Information on photoperiodicity in these classes is still meager. There is evidence that metamorphosis in the European frog *Rana temporaria* is accelerated by long daily photoperiods, although it is not clear that this is a natural timing mechanism of significance (259).

The literature on photoperiodicity in reptiles has been reviewed by Bartholomew (268). There is some evidence of photoperiodic control of reproductive cycles, invariably interacting with temperature and behavioral thermoregulation; the latter often determines the effective day length to which the animal is exposed. A further complication is the control of the effective photoperiod by the responses of the pineal eye (268). In *Xantusia vigilis*, increased daily photoperiod appears to be more important in testicular development than increased temperature (268 to 270). Fox & Dessauer (271) have brought male *Anolis carolinensis* into full breeding condition unseasonably in the fall by 60-day exposure to 18-hr. daily photoperiods; progressively greater responses were obtained with similar treatments initiated later, in winter. No evidence of a refractory period could be found. The rate of response appears to be a positive function of the daily photoperiod up to 18 hr.

## INSECTS

Photoperiodicity has been demonstrated in many species. Photoperiodic controls vary enormously in relative importance with respect to control mechanisms based on other environmental factors such as temperature, quantity and quality of food, and population density, from a primary role as in *L. decemlineata* and *H. brillians* (see Introduction) to a relatively minor role as, for example, in some monovoltine Lepidoptera (38). Recent useful reviews are those of Lees (5, 23, 24, 272), de Wilde (6), and Emme (4). Photoperiodic mechanisms are most commonly involved in the induction of diapause, including associated gonadal and metabolic effects, and in the control of body form in polymorphic species.

*Photoperiodic control of diapause.*—The photoperiod, frequently interacting with other environmental factors such as temperature, is an important factor in the induction of diapause in many species (5, 6, 7, 22 to 24, 273). Often low temperature is the only factor involved in the termination of diapause although in the moth *Dendrolimus pini* (38, 274) and the planthoppers *Delphacoides striatella* (40) and *Nephotettix bipunctatus cincticeps* (275, 276), it may be terminated by long daily photoperiods alone.

Photoperiodic induction of diapause is most frequently effected by "short days" of the order of 6 to 14 hr.; the range of photoperiods of maximum effectiveness varies extensively among species, and within a species may vary among populations as a latitudinal or other geographic function (7, 273, 277, 340). Within a population, the range of the photoperiod and the intensity of response are frequently also functions of environmental temperature, with lower temperatures usually causing an increase in frequency of response



and often extending the maximum of the photoperiod-response curve toward longer photoperiods (7, 18, 21, 33, 39, 273, 275, 278 to 282). In the cotton moth *Chloridea obsoleta*, the maximum rate of short-day induction of diapause is at about 23°C. with lower rates at both higher and lower temperatures (283). Photoperiodically induced diapause may also be modified in some species by nutritional status (5, 18, 23, 38). As examples among the Lepidoptera, short-day induced larval or pupal diapause has been demonstrated in *Acronycta rumicis* (21, 22, 32, 38, 284), *Pieris brassicae* (25, 32, 38), *Araschinia levana* (284, 285), *Grapholitha molesta* (34), *Barathra brassicae* (38, 39), *Polychrosis botrana* (37), *Antheraea pernyi* (32, 35, 286), *Dendrolimus pini* (38), and *Dipthera alpium* (32). The univoltine *Leucoma salices* and *Euproctis chrysorrhoea* are of interest in that long daily photoperiods (but not continuous light) prevent the development of diapause by a substantial fraction of the larvae; in the univoltine *E. similis*, diapause is prevented completely by 20 hr. daily photoperiods. In *Abraxis miranda* in Japan, long daily photoperiods (14 to 16 hr.) induce a long aestivation-diapause whereas photoperiods of 11 to 13 hr. induce a short prewinter diapause; short photoperiods (7 to 9 hr.) prevent either type of diapause (279, 287, 288). In *P. brassicae*, 16-hr. daily photoperiods cause the development of nondiapause pupae in the Hokkaido populations, whereas in the Honshu and other southern populations the same photoperiod causes a high frequency of aestival diapause pupae (277, 278); short daily photoperiods (<12 hr.) cause winter diapause pupae in all populations (277, 289, 290). The extensive literature on photoperiodic induction of diapause in *B. mori*, a species which has been subject to the typical selective processes of domestication, has been reviewed by Lees (5) and Morohoshi (281). Diapause occurs at an early unsegmented stage of embryonic development. In the divoltine or multivoltine strains, which produce diapause eggs, the determination of the type of development (diapause or nondiapause) is effected hormonally in early larval development of the female (51, 281). If developing eggs or early larvae are exposed to long daily photoperiods, subsequently as adult females, they produce a high proportion (70%) of diapause eggs, whereas short daily photoperiods cause the subsequent production of nondiapause eggs exclusively (5, 23, 24, 51). High temperatures enhance the diapause-egg promoting effect of long daily photoperiods, whereas low temperatures enhance the nondiapause-egg producing effect of short daily photoperiods (51, 281).

Short daily photoperiods have been shown to induce diapause also among other groups of insects. The following are examples: adult Colorado potato beetle, *L. decemlineata* (<14L) (16, 17, 18, 291 to 294); female *Culex pipiens* (<ca. 12L) (295); female *Anopheles maculipennis messeae* (295, 296); and *Diprion pini* when larvae are subjected to short days (297). An arrest of larval development is induced by short daily photoperiods and relatively low temperature in the leafhopper *N. l. cincticeps* (275, 276), and in the planthopper *D. striatella* (40). A larval diapause is induced by short daily photo-

periods (<ca. 12 hr.) in the pitcher-plant midge *Metriocnemus knabi*; the interaction with temperature is of interest since the response is maximum at 23–25°C. with a lower frequency at 12°C. and no diapause at 30.6°C. (298).

In *Aedes triseriatus*, it appears that the shortening days in autumn cause the production of a type of egg whose larvae will pupate only in long daily photoperiods thus providing a winter larva (299) by means of which the species overwinters. The winter arrest of development of the female reproductive system of the delphacid planthopper *Sternocranus minutus* is actually a preoviposition diapause induced by long daily photoperiods during the nymphal period and is terminated only by a 4-week exposure to short days; the ovarian diapause is prevented by exposure of the larvae to short-daily photoperiods (300, 301).

In several instances at least, the synchronization of the life cycle of hymenopterous parasites with that of the photoperiodically controlled life cycle of the host is based on a similar photoperiodic response of the parasite (5). Thus, short daily photoperiods have been shown to induce diapause in *Apanteles glomeratus* (25, 302), *A. spurius* (302), *Telenomus laevusculus* (302), and *Pteromalus puparum* (25) in a variety of lepidopterous hosts.

In each of the cases of photoperiodically induced diapause noted above, it either appears, or has been definitely demonstrated, that the response is to light (or dark) per se and not to a changing duration of the photoperiod. However, there is reason to believe that ecdysis to final instar and prevention of diapause in the nymph of the dragonfly *A. imperator* is caused by increasing daily photoperiod rather than by a specific duration per se (65). Norris (66) has reached the conclusion that increasing daily photoperiods cause maturation in the grasshopper *Anacridium aegypticum*, whereas constant or decreasing day lengths apparently inhibit it and cause diapause; he has also found that decreasing day lengths appear to be more effective in establishing long diapause in the red locust *Nemadaeris septemfasciatus* than fixed short daily photoperiods.

In many species in which diapause is induced by short days, there is nevertheless a definite light requirement, that is, diapause is not to be associated solely with the duration of the dark period. This is initially evident in the substantial number of species in which the frequency of induction of diapause is greater with daily photoperiods of 4 to 10 hr. than with those under 4 hr. or with continuous darkness (5, 18, 22 to 24, 32 to 34, 37 to 40, 272, 280, 284, 286, 298); the dual requirement (light and dark) has been further established with experiments with ahemeral cycles, i.e. of other than 24 hr., and in experiments with more than one photoperiod per 24-hr. cycle. Earlier evidence of this type has been summarized well by Lees (5), including the ingenious experiments of Tanaka (36, 303) on *A. pernyi* and those of Russian investigators on *A. rumicis* (21, 32, 33).

Little is known about the functional relationship between light intensity and the induction of diapause except that above relatively low intensities it



is not a function of intensity. Thus, in *B. mori* near-maximum responses were obtained at 20°C. with about 0.05 lux and about one-half maximum response at 0.04 lux (51). Larvae of *G. molesta* respond to as little as 10 lux (from incandescent lamps) incident on the outside of apples in which they are tunneling (34). Maximal intensity for larvae of *A. rumicis* is about 5 lux (284). The threshold intensity of white light in the induction of diapause in adult *L. decemlineata* is below 0.1 lux with the maximal intensity somewhat above 5 lux (304). For larvae of *M. knabi*, about 0.03 lux is maximal (298). Data on action spectra are fragmentary. The greatest sensitivity is in the blue-violet (350 to 510 m $\mu$ ) in *B. mori* (51, 305) and in *G. molesta* (34). The recent experiments of Gyeispits (72) suggest similar maxima also in *Dendrolimus pini* and *P. brassicae*. The action spectrum in *L. decemlineata* is very broad with a suggestion of two maxima at about 450 m $\mu$  and 700 m $\mu$  (292). The photoreceptors in the response systems have not been identified morphologically. Gyeispits (72) showed that they must be in the head region of *Dendrolimus pini* although Tanaka (286) demonstrated that they are not in the ocelli of *A. pernyi*. In *B. mori* the photoperiodic effect is exerted long before the development of morphologically identifiable photoreceptors. According to de Wilde (292), the eyes are not required in the response in *L. decemlineata*.

In recent years, much information, beyond the scope of this review, has been accumulated on the basic endocrine mechanisms of diapause [Lees (5, 23, 24)]. Unfortunately, only a small portion of this information concerns species with photoperiodically induced diapause although considerable attention has been directed toward *B. mori* (5, 23, 24, 281, 306). The type of egg laid by the female silk worm is determined hormonally in the pupal stage, apparently by the effect on the ovary of antagonistic hormones produced by the corpora allata and the subesophageal ganglion, a preponderance of the latter causing the production of diapause eggs and a preponderance of the former causing the production of nondiapause eggs (306). The production of diapause hormone by the subesophageal ganglion is controlled via circumesophageal connectives by neurosecretory activity of the brain (307 to 319). It is remarkable that the photoperiodic fixation of the functional endocrine response system occurs at an early stage in embryonic development. The nature of the information storage device is still unknown. Kato (305) has drawn attention to the well-established fact (51) that high temperature (25°C.) during the sensitive embryonic period makes long photoperiods unnecessary for the development of diapause-egg-producing females; he has proposed a hypothetical scheme in which the necessary excited state for the level of metabolism required for production of diapause eggs may be attained by photoactivation of demonstrated riboflavin derivatives or by the effect of elevated temperatures on a critical enzymatic reaction. De Wilde & Stegwee (320) have shown that allatectomized adult *L. decemlineata* develop diapause which is similar to that in normal adults subjected to 10 lux; this is consistent with the hypothesis of an antagonism between the corpora allata and the subesophageal ganglion, as in *B. mori*.

*Photoperiodic control of form in polymorphic species.*—This fascinating aspect of photoperiodicity in insects has been reviewed recently by Lees (24). Considerable attention has been directed toward the aphids which display a variety of complex polymorphic cycles and an almost perplexing variety of control mechanisms. There is now sufficient experimental evidence to show that short daily photoperiods, invariably interacting with other environmental factors, cause, in several species, a cessation of production of parthenogenic virginoparae and subsequently the production of oviparous females, of males, and the consequent resumption of sexual reproduction (52 to 54, 321 to 324) characteristic of the populations in fall or winter. Among the psyllid homopterans there are interesting photoperiodic controls of form and induction of diapause. For example, in the pea sucker *Psylla pyris*, short daily photoperiods (4 to 12L) invoke the development of a high percentage of the morphologically distinctive winter forms and in these a non-obligate reproductive diapause (325, 326). Also, among the Homoptera, Müller (327, 328) has shown in the jassid leafhopper *Euscelis plebejus* that there is a photoperiodically controlled seasonal dimorphism in body size, pattern, and in form of the penis; the spring form (formerly regarded as a distinct species) is induced by short daily photoperiods (ca. 8L) and the summer form by long daily photoperiods (ca. 16L); temperature and other environmental factors only modify the response. All five larval stages are photoperiodically sensitive; threshold intensity appears to be between 5 and 8 lux. There is a photoperiodic control of body size in *S. minutus*, short days inducing a smaller body size (300, 301). Among the Lepidoptera, the marked seasonal dimorphism in *A. levana* is photoperiodically controlled; eggs from which the summer form (*prorsa*) develops come from females treated as larvae with long (16 to 20L) daily photoperiods whereas eggs from which the spring form (*levana*) develops come from females treated as larvae with ca. 8L, which also induces pupal diapause (284, 327).

#### MITES

Investigations on photoperiodicity in this group have been confined to the red-spider mites [Lees (5, 23, 24, 272)]. The fruit tree red-spider mite *M. ulmi* lays two types of eggs, a winter or diapause egg and a summer or nondiapause egg. The character of the egg is determined by the female; winter-egg- and summer-egg-producing females are morphologically indistinguishable. The development of "winter" females may be caused by short days (6 to 13L), low temperature, or restricted food. In the photoperiodic induction of "winter" females, maximal intensity is of the order of 10 to 20 lux and the maximum in the action spectrum is in the blue region. Greatest sensitivity is in the deutonymphal instar but even adult females can be "switched" (329). Both the light and dark periods appear to be involved in the determination process (330). In *Tetrarhynchus telarius*, adults winter in diapause which can be induced by short daily photoperiods and is broken by chilling (329). Bondaryenko (331) has demonstrated that short daily photoperiods (<14L)

induce diapause in male *T. urticae*, but diapause will develop only if short photoperiods occur before the adult stage.

#### OTHER INVERTEBRATES

Although it is probable that photoperiodicity may be widespread among the remaining invertebrate groups, there are actually few data to support such an assumption (1, 56). Among the mollusks, Jenner (332) has presented evidence indicating that oviposition is, at least in part, photoperiodically controlled in the snail *Lymnaea palustris*, but Mori & Matutani (333) concluded that no such mechanism occurs in two Japanese land snails, *Bradybaena similaris* and *B. sieboldiana*. Among the crustaceans it appears that photoperiodic mechanisms are involved in the control of the ovarian cycle and secondary sex characteristics (334), in the molting cycle (335) of the crayfish *Cambarus virilis*, and in the control of the molting cycle of *Palaeomonetes paludosus* (336). Bliss (337) showed that the essential growth processes which precede molt in the decapod crustacean *Gecarcinus lateralis* are inhibited by continuous light and that this inhibition is probably mediated directly through the neurosecretory system. However, caution must be exercised in regarding this as a photoperiodic mechanism in the true sense of the word since the photoperiod of the animal is a behavioral function in a manner similar to that noted above for reptiles. Of interest are the ingenious experiments of Hauenschild (338) on the nereid polychaete worm *Platynereis dumerilii*. This species appears to have a photoperiodic response mechanism which has such a high sensitivity that the light of the full moon is above threshold intensity. This, together with day length, exerts a "continuous light effect" which is necessary for an essential stage of development that is then followed by the shedding of sex cells, thus providing a photoperiodic basis for a lunar biologic cycle.

#### GENERAL CONCLUSIONS

Among many mid- and high-latitude species, the changing duration of the daily photoperiod serves as a biological calendar which, in conjunction with adequately evolved internal mechanisms, allows the animal to "anticipate" seasons and to begin appropriate physiologic preparations in advance. This allows the temporal placement of major physiologic events in the seasons most advantageous to the survival of the species. Among the functions known to have basic photoperiodic controls, at least in some species, are reproduction and molting in mammals; reproduction, molting, and migration in birds; reproduction and migration in fish; and in insects and mites, diapause including associated metabolic and reproductive changes, and control of body form in polymorphic species. The relative importance of the photoperiodic timer varies enormously among species. In some species the photoperiodic effect is primarily one of monitoring the phase and frequency

of an internal rhythm with an approximate annual frequency, whereas in other cases the cyclic function appears to be a directly dependent function of photoperiod. Among poikilotherms there is obviously a large variety of interactions with temperature. Although some progress has been made in the identification of the components and performance characteristics in a few species of mammals, birds, fish, insects, and mites, knowledge has not yet reached a satisfactory state for any species.

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<sup>1</sup> In transliterating the Russian references, the author has used the following system with the vowels: e=ye, u=i, ю=yu, я=ya, ъ=y, а=a, о=o, э=e.

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# PHYSIOLOGICAL ASPECTS OF AGING IN MAN<sup>1</sup>

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## INTRODUCTION

*Definitions.*—Aging is a process that takes place over the entire life span of an organism. Previous volumes of the *Annual Review of Physiology* have dealt with age changes that occur during the early part of life. Thus, a number of reviews are available by Barth (1), Needham (2), and Spratt (3) on developmental physiology. Various aspects of growth have been reviewed by Klein (4) and Russel & Wilhelmi (5) as well as Sontag & Garn (6). The present review will be concerned with the physiological changes that take place in the organism between the attainment of maturity and death. The systematic study of this period of the life span (gerontology) has expanded markedly during the past ten years so that there is a formidable volume of literature available for review [Shock (7)] which continues to expand at a rapid rate [Shock (8)]. At the present time, there are approximately fifteen special journals in the field of gerontology.<sup>2</sup> This review will be limited to a consideration of physiological changes with age in various organ systems, with primary reference to man. Space limitations will prevent review of the basic biology of aging, including important aspects of age changes in cellular physiology.

*Aging and disease.*—In man, old age is commonly associated with disease. However, aging cannot be regarded as synonymous with disease. This review will not be concerned with disease processes as such, but rather with the physiological framework within which disease may develop. To this end, only studies made on ambulatory subjects will be considered. Primary emphasis will be given to studies in which the subjects have failed to show clinical or laboratory evidence of present or past disease of the organ system under study. It is obvious that no examination can completely exclude the presence of a disease process, but careful examinations can reduce significantly the incidence of disease in the sample studied. It may be that twenty years hence many of the observations that we presently regard as "aging"

<sup>1</sup> The systematic survey of literature for this review was concluded in May 1960. A few pertinent references appearing in June were included.

<sup>2</sup> The major journals, which specialize in gerontology and may contain articles of physiological interest, are listed with dates of first year of publication: *Acta Gerontologica Japonica* (1929); *Acta Gerontologica Milano* (1951); *Archives of Gerontology, Japan* (1936); *Geriatrics* (1954); *Geriatrics* (1946); *Gerontologia* (1957); *Gerontologia Clinica* (1959); *Giornale di Gerontologia* (1953); *Journal of the American Geriatrics Society* (1953); *Journal of Chronic Diseases* (1955); *Journal of Gerontology* (1946); *Revista de Geriatria* (1956); *Revue Française de Gerontologie* (1952); *Rivista di Gerontologia & Geriatria* (1952); *Zeitschrift für Altersforschung* (1938).

may prove to be only the signs of early disease, which we cannot now identify. Nevertheless, the observations remain valid as an indication of age trends in a population.

*Methods of study.*—Aging can be studied only in terms of changes that take place in some function, trait, or characteristic with the passage of time. Ideally, measurements should be made at different points of time in the life cycle of a single individual in order to study the aging of the trait. However, this method is impractical where the life span of the animal is as long or longer than that of the experimenter, and is impossible for studies that require sacrifice of the individual animal. Consequently, most of the data available are really concerned with age differences between groups. This method is fraught with many sampling problems, especially in the case of studies on man. When observations on a group of 70-year-olds are compared with those on 30-year-olds, no one knows what the 70-year-olds were like when they were 30. Furthermore, the 70-year-olds have been selected for longevity already. In addition, they have been exposed to different environments and may be quite unlike the 30-year-old of today when he attains the age of 70 in the year 2000.

In spite of these difficulties and limitations, it is possible to identify age differences in a number of physiological variables. It is the goal of this review to examine the broad generalizations that can be made about the physiological aspects of aging and to offer illustrations of observational data rather than to give a detailed catalogue of age differences in each specific organ system. Reference will be made to a few key publications appearing before 1957, but major emphasis will be placed on the recent literature. Although there is no single volume which deals with the physiological aspects of aging, the books by Binet & Bourlière (9), Birren (10), Bürger (11), Comfort (12), Grmek (13), Lansing (14), Nikitin (15), Shock (16), Stieglitz (17), and Thewlis (18) contain chapters which deal with the aging of various physiological characteristics.

#### MAINTENANCE OF THE STABILITY OF THE INTERNAL ENVIRONMENT

The maintenance of the cellular environment within relatively narrow limits is required for survival. The circulating blood represents the nearest approach to a characterization of the internal environment in man. Old subjects retain a remarkable capacity to maintain the chemical and physical composition of the blood under basal or resting conditions.

*Blood volume.*—Using the dye dilution technique (T-1824), Cohn & Shock (19) found no significant age changes in plasma or blood volume. These results have been confirmed by Schröder & Börner (20) and by Smith (21) using  $I^{131}$ -tagged albumin and  $Cr^{51}$ -tagged red cells, respectively. The range of individual variation in blood volume is large, so it is not surprising that Sklaroff (22) and Reeve *et al.* (23) have reported a small reduction in blood and plasma volumes in small groups of elderly hospital patients. Within the limits of present analytical methods and the wide range of biological varia-

bility, blood and plasma volumes per kg. body weight are uniform throughout the life span.

**Erythrocytes.**—In the absence of disease, the elderly subject is able to maintain the number of red cells and hemoglobin content of the blood within normal limits [Shapleigh, Mayes & Moore (24)]. However, red cells produced by aged subjects are, on the average, larger in diameter than those produced by the young [Schlomka & Peschel (25)]. Spriggs & Sladden (26) reported an increase in red cell diameter of  $0.06\ \mu$  per decade after the age of 40. The erythrocyte increases in resistance to mechanical trauma with the age of the subject. Schlomka & Christiani (27) rotated red cells with glass beads under standard conditions and reported hemolysis of 10.5 per cent of the red cells from subjects 15 to 29 years of age compared with 6.2 per cent hemolysis in red cells from 60- to 83-year-old subjects. Using the standard clinical technique, Olbrich (28), as well as Bornemann & Knobloch (29), reported an increased osmotic fragility in red cells from old people. When the more carefully controlled methods described by Parpart *et al.* (30) were used in our laboratory, no significant age differences in osmotic fragility were found.

In a population survey of 857 subjects, which included 88 per cent of the population, aged 60 to 70, on the island of Bornholme, Dencker & Felbo (31) found a mean sedimentation rate of 13 mm. per hr. in males and 21 mm. per hr. in females, which is not significantly different from other values reported on young adults. Gillum & Morgan (32) observed sedimentation rates of 10 to 15 mm. per hr. in males and 18 to 24 mm. per hr. in females aged 50 to 80. The 80+ group showed slightly higher values.

Thus, the older individual is generally able to produce an adequate supply of red cells, but there may be subtle changes in the character of the cells produced.<sup>3</sup>

**Plasma proteins.**—In addition to serving a nutritive function, the plasma proteins play an important role in regulating the osmotic pressure of the blood. In accord with previous reports, Eckerstrom (33), Parfentjev & Johnson (34), and Zerman & Franca (35) found no significant change with age in total plasma protein among institutionalized elderly subjects. Morgan, Murai & Gillum (36) observed the same serum protein levels (6.4 to 6.7 gm. per 100 ml.) among 573 men and women, 50 to 80 years of age, all living in their own homes in San Mateo County, California, except for 44 of the men living in a county home. Although the values were slightly lower (10 to 15 per cent) than those reported by others on young adults, the difference was not statistically significant.

When more detailed fractionations of serum proteins are made, age differences appear. Electrophoretic analyses showed a small but significant reduction in albumin [Chesrow *et al.* (37)] and a rise in globulin [Prusik (38); Nöcker (39)], fibrin [Doberauer *et al.* (40)], and fibrinogen [Richter (41)].

<sup>3</sup> The extensive literature on changes in the red cell over its individual life of 90 days cannot be reviewed because of space limitations.

The albumin to globulin ratio diminishes with age [Ries (42)]. Walter & Haurowitz (43) prepared "young" and "old" proteins by withdrawing plasma from rats 8 hr. and 93 hr. after the injection of  $S^{35}$ -labeled amino acids from yeast protein hydrolysates. The "young" and "old" proteins were then injected into other rats and the rate of disappearance of radioactivity was followed. No significant difference in half life was observed. The authors concluded that the "age" of a protein molecule had no influence on its rate of breakdown. These are ingenious experiments which should be repeated with increasing "ages" of the proteins.

*Plasma electrolytes.*—The ability to regulate the electrolyte composition of the plasma under basal or resting conditions is retained into advanced old age. Lippi & Malerba (44) found no age differences in sodium or potassium concentrations in the plasma. Both Hamilton *et al.* (45) and Vallecorsi & Dominici (46) reported uniform levels of inorganic P of the plasma between the ages of 20 and 80. Shock & Yiengst (47) found no significant age changes in  $pH_a$ ,  $[BHCO_3]_a$  or  $pCO_2$  under resting conditions.

*Blood glucose.*—Although a number of early studies have reported a slight rise in fasting blood sugar levels, more recent studies by Smith & Shock (48), as well as Gillum, Morgan & Williams (49), have failed to find any significant changes with age.

*Blood urea and nonprotein nitrogen.*—There is a slight increase in uric acid, creatinine, nonprotein nitrogen, and urea at ages 80 and above. Lewis & Alving (50) found a significant positive correlation between age and blood urea nitrogen. However, their data indicated uniform mean values of about 12.5 mg. per cent between the ages of 40 and 70, with a rise to 18 mg. per cent between the ages of 70 and 90. A few subjects (aged 70 to 90) had blood urea nitrogens as high as 30 mg. per cent. More recently, Morgan, Murai & Gillum (51) have reported a similar rise amounting to about 28 per cent at later ages in nonprotein nitrogen, but creatinine and uric acid levels did not change significantly. In fact, the average levels fell slightly at advanced ages.

*Blood cholesterol and lipoproteins.*—The original observation of Keys (52) that average blood cholesterol levels in males rise from age 20 to a maximum at age 55–60 and then fall, has been confirmed by many reports. In a massive co-operative study involving 10,379 normal subjects living in different parts of the United States, Lewis *et al.* (53) found a general rise in the mean levels of cholesterol and lipoprotein classes in both sexes up to the fifth decade of life.<sup>4</sup> In females, the rise continued up to the seventh decade so that, at higher ages, the values for females exceeded those of males. In males, all lipoprotein classes reached a maximum in the fifth decade and then decreased. Although part of the decrease in mean values in males may reflect selective mortality from coronary artery disease among subjects with high

<sup>4</sup> A detailed statistical analysis of these observations has been made by Tamplin & Tandy (54).



values, Keys (52) has found a reduction in values in the same subject after age 55. Acheson, Hemmens & Jessop (55) have also observed a reduction in blood cholesterol after the age of 65 in a community residing group of pensioners in Ireland. Schaefer, Adlersberg & Steinberg (56) have also found a sex difference in the pattern of age changes in phospholipids of the blood. In males, levels rose between the ages of 20 and 32 and then remained constant through age 60. In females, there was no change up to age 30, but thereafter there was a uniform rise through age 60.

*Vitamin levels in blood.*—Kirk (57) has reviewed the status of age changes in blood level of a number of vitamins. Vitamin A, riboflavin, and thiamine levels do not change with age. Ascorbic acid levels showed a small but significant fall [Morgan, Gillum & Williams (58)]. Gaffney *et al.* (59), as well as Chernish *et al.* (60), have observed a small but statistically significant fall in plasma vitamin B<sub>12</sub> levels with age. In view of the extremely wide variation between individuals at any age, it is doubtful whether the age decrements have physiological significance.

#### PATTERNS OF AGE DIFFERENCES

*Types of differences.*—Present knowledge does not permit the conclusion that there is a general aging process. Although some writers, such as Bürger (11), emphasize the unitary character of aging, observations show that there are wide differences between the age changes in different physiological systems in man. Ultimately we must have measurements of different traits made on the same individual over a period of time, but for the present we can only compare age differences among different traits on the basis of average curves. From such data, it is clear that aging progresses much more rapidly in some traits than in others.

As indicated in the previous section, some traits do not change significantly with age. Other characteristics reach their maximum relatively late in life and then fall. The plasma levels of cholesterol and lipoproteins previously described represent one example. In females, the mean free acidity in the stomach, following an Ewald test meal, rises at a decreasing rate to the age of 30 and then remains constant up to the age of 80. In males, gastric acidity rises to considerably higher values by the age of 25, remains at high levels until the age of 45, and then falls linearly until at age 80 it is the same as in females [Vanzant *et al.* (61)]. The incidence of achlorhydria also increases with age, especially in males after age 40.

Another pattern of age changes is a rapid reduction in function during early adulthood with a minimum of change during later life. An example of this pattern of aging is the reduction in range of accommodation of the eye, which falls from about 13 diopters at age 8 to 1 diopter at age 55, with only a small change thereafter [Friedenwald (62)].

However, the most general pattern of age changes is a linear decrement in function beginning about the age of 30 and continuing throughout life. Al-





though linear decrements are common to a number of different functions, the slopes of the regression on age vary considerably (from 10 per cent to 60 per cent between the ages of 30 and 90).

*Cardiovascular system.*—The cardiovascular system holds a key position in the interpretation of age changes throughout the body, since an adequate blood supply is essential for any organ system or cell to meet the demands placed upon it. In man, the disease process of arteriosclerosis plays an important role in many of the functional impairments which accumulate with age. However, it is erroneous to assume that all changes are due entirely to sclerotic changes in blood vessels and the mechanical impediment to blood flow, since individuals vary widely in the extent to which they suffer from the disease (arteriosclerosis), and age changes may be observed in the absence of detectable arteriosclerosis.

*Cardiac output.*—Brandfonbrener, Landowne & Shock (63), using the dye dilution technique, found a fall in resting cardiac output from 6.5 to 3.8 l. per min. between the ages of 25 and 85. The stroke volume fell from 85 to 60 ml. per beat over the same age interval. Correction of these indices for surface area reduced the age decrement slightly, but it was still highly significant. Although the method may be questioned for comparisons between individuals, there was a similar downward trend in cardiac output estimated from ballistocardiographic records [Tanner (64)].

*Blood pressure.*—A statistical analysis of resting blood pressure measurements in a sample of 79,757 apparently healthy subjects, aged 20 to 106 years, by Lasser & Master (65) indicated a gradual rise in systolic pressure (from 123 to 145 mm. Hg) up to the age of 70, with no further change between 70 and 100 in males. In females, the systolic pressure increased from 116 to 158 mm. Hg between the ages of 20 and 75 years and then gradually fell to reach 149 mm. Hg at age 100. Diastolic pressure did not change significantly in either sex (75 to 85 mm. Hg). With increasing age, the standard deviation and the skewness of the distributions of pressure increased.

In a sample of 17,900 males and 26,281 females living in Bergen, Norway, both systolic and diastolic blood pressures increased progressively with age from 15 to 75 years in both sexes according to the findings of Bøe, Humerfelt & Wedervang (66). Industrial and rural workers of Delhi, India, failed to show any rise in systolic or diastolic blood pressures, between the ages of 23 and 62 years, although individuals of high socio-economic status from the same area showed a progressive rise in systolic pressure of about the same order of magnitude as shown by U. S. males [Padmavati & Gupta (67)]. Brown, McKeown & Whitfield (68) analyzed blood pressure measurements made by physicians on 1045 males, aged 60 to 69. In this population, systolic and diastolic blood pressures tended to rise as economic and social circumstances became less favorable. There was no evidence that at this age blood pressure was related to the physical or mental demands of employment. It must be concluded that social and cultural conditions can influence age changes in a population.

*Cardiac work and performance.*—In experiments where simultaneous measurements of intra-arterial blood pressure and cardiac output were made, it was possible to compute approximations of left ventricular work and stroke power. Landowne, Brandfonbrener & Shock (69) observed a significant reduction in both work and power with increasing age in 67 males, aged 23 to 82, who had been carefully screened to exclude individuals with clinical evidence of cardiovascular disease. The decrease in left ventricular work was largely the result of a diminished cardiac output. The reduced power or rate of work was a reflection of the longer duration of systole in the cardiac cycle.

Additional evidence of diminishing power of the heart may be found in ballistocardiographic tracings. Taylor & Walker (70) constructed a special ballistocardiograph which permitted recordings under high-frequency conditions used by Starr or low-frequency critically damped conditions used by Nickerson without moving the subject from the bed. In agreement with previous studies, they found lower I-J amplitudes in a group of 57 men, aged 50 to 59, than in 51 young men, aged 18 to 26. In their experience, the low-frequency critically damped apparatus was more sensitive to the effects of age than the high-frequency apparatus. Starr & Hildreth (71) recorded a second ballistocardiogram after an interval of 10 to 14 years in 80 normal subjects aged 20 to 60. The I plus J amplitude diminished by roughly 50 per cent over the age span tested. The authors interpret these results as indicating a reduction in the force of ejection and a slowing of the contractile response of the ventricle. However, the acceleration measured by the ballistocardiograph depends also on the vascular impedance, which includes the central volume to be accelerated, distensibility of vessels, and frictional resistance, all of which may also undergo changes with age. In fact, Honig & Tenney (72), using an aperiodic ballistocardiograph, claim that beginning at age 40 there is a progressive shift of the frontal plane vector toward the transverse axis of the body and that the reaction force actually remains constant throughout adult life.

*Circulation time.*—The speed of circulation of the blood, as measured by decholine, lobeline or by the mean transit time of dyes injected for the estimation of cardiac output, shows a significant slowing with age. The mean transit time of T-1824 from the antecubital vein to the brachial artery increased from 19.0 sec. to 28.7 sec. between the ages of 23 and 82 years [Landowne, Brandfonbrener & Shock (69)]. Herbeuval *et al.* (73), using the respiratory response to an intravenous injection of lobeline as an index, found an increase in circulation time from 7 to 16 sec. between the ages of 60 and 95 in a group of 100 subjects.

*Electrocardiogram and phonocardiogram.*—The P-Q, P-R, and QT intervals of the electrocardiogram show a slight tendency toward prolongation in elderly subjects. The effects are more marked in subjects with slow heart rates. The voltages of P, R, and T waves decline after the age of 60. The mean electrical axis of the P wave, which is deviated to the left at birth, becomes vertical by the age of 40 and then shifts progressively to the left as

age advances. The mean electrical axis of the QRS complex behaves in an opposite manner, indicating a more horizontal position of the heart at advanced ages [Simonson & Keys (74); Mezzasalma & Morpurgo (75); Michel (76)]. Fisch *et al.* (77) correlated electrocardiograms of 500 ambulatory patients 70 years of age or older with clinical findings. Abnormal tracings were twice as common in the patients with heart disease as in those without. However, only 38 per cent of the abnormal tracings were accompanied by detectable heart disease.

Simonson & Keys (78) carried out a vector analysis of conventionally recorded electrocardiograms and found that both the QRS and T vectors were rotated more anteriorly (larger azimuth angle), were smaller in magnitude, and had smaller angles between the vectors in old than in young men. Burch, Golden & Cronvich (79) recorded spatial vectorcardiograms in 159 men and 64 women, aged 20 to 68, with a cathode-ray oscilloscope and the equilateral tetrahedral reference system. The records included projections of the sVCG on the frontal, right, left, and superior planes of the equilateral tetrahedron, and stereoscopic views of these projections, as well as left sagittal projections of each plane. Wire models of each sVCG were constructed for proper orientation of the loop in space. Records from 223 normal subjects, aged 20 to 68, were analyzed. Distortion of the QRS<sub>E</sub> loops, or pronounced irregularity in the time course of the wave of depolarization was observed with increasing frequency at advancing age.

At present, it is not possible to assess the relative importance of alterations in the electrical activity of the heart and anatomical or positional changes in producing the observed age changes in the electrocardiogram.

Aravanis & Harris (80) have reported a slight increase in the duration of heart sounds recorded electrically in a group of 100 subjects, aged 60 to 108, with clinically normal hearts.

*Blood vessels—structure.*—With advancing age, the chemical composition of the larger vessels changes. Lansing (81) has reported an increase in specific gravity, calcium content, and proportion of amino acids containing free carboxyl groups in the aorta with advancing age. There was an increase in aspartic and glutamic acids and a decrease in glycine, proline, and valine in the elastin. The deposition of collagen increases in the intimal and medial layers of blood vessels.

*Blood vessels—pulse wave velocity.*—A number of investigators have confirmed the original observations of Hallock (82) that the pulse wave velocity, determined at diastolic pressure, increases significantly with age [Karnbaum (83); Michel & Hartleb (84); Dal Palù *et al.* (85); Landowne (86)]. In the aorta, pulse wave velocity increases, on the average, from 4 m. per sec. at age 20 to 10 m. per sec. at age 65 whereas, in the carotid-radial artery, it increases from 5 to 9 m. per sec. [Hallock (82)]. In the series of subjects reported by Landowne (86), there was no increase in pulse wave velocity in the brachial-radial segment beyond the age of 50. In a group of 200 trained

males who continued to be active in sports, the age increment in pulse velocity was from 5.5 to 7.5 m. per sec. between the ages of 20 and 65 [Mellero-wicz & Petermann (87)]. Since the pulse wave velocity is highly dependent on intravascular pressure [Landowne (88)] and pressure-volume curves of the human aorta are nonlinear, conventionally recorded pulse wave velocities cannot be used directly as indices of alterations in the elastic properties of blood vessels. Cope (89) has developed equations, assuming a parabolic relationship between pressure and volume, which permit calculation of parameters related to elasticity. From an analysis of existing data, a significant change in elasticity of the aorta with age was found. Using an ingenious technique of superimposing an experimentally produced pressure wave on the normal intravascular pressure wave, Landowne (86) found that the velocity of the experimental pressure wave also increased with age at low pressures (diastolic) but, at high pressures (systolic), the age difference was small. Thus, in older subjects, the artery behaves as if its fibers were initially more completely extended than in the young.

*Regional blood flow.*—Calculations of peripheral resistance, from simultaneous measurement of pressure and flow, show that resistance increases with age in all capillary beds. The age change is not uniform for all organs.

Ries (90) observed a decrease in capillary permeability in old age. The estimate was based on the change in albumin to globulin ratio and hematocrit of the blood following artificial interruption of the circulation (30 min. at 60 mm. Hg).

The vasomotor response of peripheral blood vessels to both heat and cold shows a reduction with age. However, the primary change was in the speed of response rather than the final degree of vasodilation or constriction attained [Landowne, Silver & Silverstone (91); de Crinis *et al.* (92)]. Allwood (93) found no difference in resting blood flow in the foot and calf between 20 men, aged 70 to 82, and 10 male students, aged 18 to 24, when estimated plethysmographically. Maximal flow during reactive hyperaemia was diminished in the elderly and more in the foot than the calf. In contrast, Ring, Kurbatov & Shannon (94), also using a plethysmograph, found that blood flow through the finger fell from 4.77 to 2.76 ml. per 10 ml. finger volume per min. between the ages of 40 and 60. Studies by Hellon and his associates (95, 96) lead to the conclusion that peripheral blood flow in the arm continues to rise up to the age of 45.

There is a rapid fall in the circulation to the brain from childhood through adolescence followed by a more gradual but progressive reduction throughout the remainder of life. According to mean curves reported by Kety (97) and Sokoloff (98), cerebral blood flow falls from 55 to 40 ml. per 100 g. per min. between the ages of 25 and 95. However, preliminary results of a study based on older noninstitutionalized individuals still living in the community showed no age differences in blood flow or calculated cerebral vascular resistance [Sokoloff (98)].

Kidney blood flow, determined by the clearance of diodrast or para-aminohippuric acid, falls by about 60 per cent between the ages of 25 and 90 [Shock (99); Uhlmann (100)].

To settle the question of whether aging is accompanied by a redistribution of blood flow among different organs, we need simultaneous measurements of cardiac output and organ flows in subjects of different ages. Lacking this information, we must rely on comparisons between average measurements made at different times on different people. Examination of average curves leads to the hypothesis that the perfusion of all organs does not fall in direct proportion to the reduction in cardiac output. As indicated above, the age reduction in blood flow to the kidney is greater than the fall in cardiac output or flow to the brain.

Regional differences in the effect of age on blood flow might be regarded as reflections of differences in the degree of structural changes, such as arteriosclerosis in the vascular beds involved. However, this hypothesis is not in accord with observations which indicate that the reduction in blood flow in certain vascular beds is produced by a functional vasoconstriction that can be reduced by vasodilating agents. For example, the intravenous administration of a vasodilating agent (pyrogen) produced proportional increases in renal blood flow in both young and old subjects [Shock (99)]. The increase in flow was the result of decreasing renal arteriolar constriction. In the aged subject, under resting conditions, a functional increase in vascular resistance reduces blood flow to the kidney, which may make a greater proportion of the cardiac output available for perfusion of other organs, such as the brain.

*Renal function.*—Along with the gradual reduction in renal blood flow, there is a similar reduction in glomerular filtration rate, maximum excretory rates for diodrast and para-aminohippuric acid, and maximum resorptive rate of glucose [Shock (101, 102); Frischauf, Krammer & Schmidt (103)]. Van Pilsum & Seljeskog (104) obtained a fairly close agreement between 24-hour endogenous creatinine and short-term inulin clearances reported in the literature in a small series of males. The filtration fraction, calculated as the ratio of glomerular filtration rate to renal plasma flow, may rise slightly at ages of 70 and above [Frischauf, Krammer & Schmidt (103)]. In old subjects with elevated diastolic blood pressures (over 95 mm. Hg), the filtration fraction is usually elevated [Olbrich *et al.* (105); Stewart (106)].

The reduction in renal function results in higher plasma concentrations of certain drugs, such as penicillin, when similar doses are administered to old and young subjects [Leikola & Vartia (107)]. Mathiesen & Trolle-Lassen (108) found that the age decrement in penicillin clearance was significantly greater than the fall in inulin clearance, a reflection of the associated fall in tubular activity with age which contributes to the excretion of penicillin.

The tubules remaining in the aged kidney are still capable of responding qualitatively to physiological stimuli, but the responses are quantitatively reduced in the old. The original observations of Miller & Shock (109), that the inhibition of a water diuresis following the administration of pitressin

was diminished in the aged, have been confirmed and extended by Tassinari & Pin (110) who found an abrupt fall after age 70 in concentrating ability of the kidney when stimulated by posterior pituitary extract. Lindeman, van Buren & Raisz (111) reported a significant fall with age in the maximum osmolarity of the urine after a 24-hour period of water deprivation. Van Zonneveld (112), in a population survey of the Netherlands involving 1562 men and 1510 women over age 65, found an increasing incidence of albumin in the urine. However, even at age 85 and above, 68 per cent of both men and women showed no albuminuria, glycosuria, or urobilinuria.

The slopes of the age regression for all renal functions tested are strikingly similar (about 6 per cent per decade) which, with the relatively high intercorrelations between different functions, leads to the hypothesis that with advancing age nephrons are lost as units. Other evidence leads to the conclusion that the nephrons remaining in the aged show physiological impairments.

*Pulmonary function.*—Lung compartments and bellows function of 135 male subjects, evenly distributed between the ages of 20 and 90 years and selected by specific criteria designed to exclude acute and severe chronic pulmonary pathology, showed a significant increase in the fixed lung space (residual volume) at the expense of mobile lung space (vital capacity) with increasing age. The average reduction of 17.5 ml. per  $M^2$  per year in vital capacity and increase of 13.0 ml. per  $M^2$  per year in residual volume were significant. The decrease in total lung capacity per  $M^2$  body surface area was not significant [Norris *et al.* (113); Horn & Schindowski (114)]. The average values of residual volume, expressed as percentage of total lung capacity, increased from 24.4 per cent in young men to 31.8 per cent in 148 men aged 47 to 57 years [Brozek (115)]. The fall in maximum ventilatory capacity (MVC) with age has been confirmed by Sartorelli & Ingegnieros (116) and Carstens *et al.* (117). The reduction in MVC resulted primarily from the inability of older subjects to increase their ventilation rate, rather than from reduced tidal volumes under the conditions of the test [Norris *et al.* (113)]. Carstens *et al.* (117) found a higher correlation between MVC and occupation than between MVC and age.

Although relatively few subjects were studied, Frank, Mead & Ferris (118) reported a decrease in dynamic compliance of the lung in elderly subjects. Pierce & Ebert (119) recorded pressure-volume diagrams in 15 subjects aged 65 to 86, using the intraesophageal balloon technique. In these subjects, the static intrathoracic pressure was less negative for any given level of lung inflation than in young controls, and the pressure-volume diagram was curvilinear. Airway resistance did not increase with age, in contrast to patients with obstructive pulmonary emphysema. The authors conclude that aging produces a primary change in the elastic properties of the lungs.

Wide individual differences and unreliability of methods make it extremely difficult to assess age differences in the diffusing capacity of the lung. Ogilvie *et al.* (120), as well as Bates & Christie (121) and McGrath & Thom-

son (122), have reported significant reductions in diffusion capacity of the lung to carbon monoxide in old men. Hanson & Tabakin (123) measured carbon monoxide diffusing capacity during exercise in normal males, aged 20 to 60, and found significant decrements only between the 20- to 29-year-old subjects and those 50 to 59.

When individuals were subjected to the combined stress of severe exercise and anoxia, the maximum diffusing capacity of the lung for oxygen diminished by 7.7 ml. per min. per mm. mean oxygen gradient per decade between 20 and 70 [Cohn *et al.* (124)].

In general, the age changes in pulmonary function are similar in kind, but much less than those found in emphysema.

*Muscular system.*—Aging is associated with a linear decrease in muscle strength beginning by age 35 [Welford (125)]. Bajusz (126) recorded myographic fatigue curves and reported diminished force of contraction and earlier onset of fatigue in old than in young subjects. Individuals who participated in sports showed less decline with age than sedentary subjects.

*Basal metabolism.*—Basal metabolism, when calculated as calories per square meter per hour, shows a gradual decrement with age [Shock & Yiengst (127)]. However, it cannot be inferred that there is a reduction in the rate of cellular metabolism, since the age decrement disappears when other indices of the amount of functioning protoplasm, such as total body water content, are used instead of surface area [Shock (128)]. It is highly probable that the apparent reduction in basal metabolism is only a reflection of tissue loss with advancing age.

#### AGE DIFFERENCES IN RESPONSES TO STRESS

Age differences become especially apparent when the individual is subjected to a stress that produces physiological displacements. Functions which show no age differences when measured under basal or resting conditions often show significant differences in the degree of displacements and the rate of recovery following experimental displacements. For example, the rate of recovery of the blood glucose level, following the intravenous administration of glucose, is slower in old than in young subjects. Furthermore, the simultaneous administration of insulin had a greater effect on the rate of disappearance of glucose from the blood in the young than in the old [Silverstone *et al.* (129)].

*Exercise.*—Norris & Shock (130) have recently reviewed the physiology of exercise in the aged. In addition to decrements in muscle strength and endurance, the ability to maintain co-ordinated muscle work declines with age. One of the most striking characteristics of old people is the inability to accelerate heart rate under conditions of heavy work [Robinson (131); Åstrand (132)]. Young men were able to increase their heart rates to 200 beats per minute, whereas older men showed maximum increases to only about 160 beats per minute (average) with heavy exercise. Åstrand, Åstrand



& Rodahl (133) were unable to raise the ceiling for maximum heart rate by having older subjects breathe oxygen during exercise.

With light exercise, old subjects show greater displacements in heart rate and blood pressure and require a longer time for recovery than do young [Norris, Shock & Yiengst (134)].

Although resting ventilation volume does not change significantly with age, under exercising conditions old subjects show a much greater increment in pulmonary ventilation to achieve a given increment in oxygen uptake than do young [Norris & Shock (135); Sartorelli & Scotti (136)].

The average maximum oxygen uptake falls from approximately 3.5 l. per min. at age 20 to 1.5 l. per min. at age 80. Similar age decrements have been observed in individuals (Dill, Dawson) who have performed the same exercise at different age periods [Simonson (137)]. When submaximal exercise is continued long enough to permit metabolic equilibrium, there is little evidence for age differences in oxygen uptake except at very low levels of work [Norris & Shock (135)]. However, Hollmann, Venrath & Valentin (138) have found that old subjects require more time to establish a steady state than do young.

The rate of recovery of heart rate, blood pressure, oxygen consumption, and carbon dioxide elimination following exercise is slower in old subjects than in young.

*Response to postural changes.*—When passively tilted from supine to the upright position, older subjects showed a greater decrease and slower recovery of diastolic blood pressure and a smaller increase in heart rate than did young [Norris, Shock & Yiengst (134)].

*Antibody formation.*—The titers of anti-A and anti-B agglutinins reach a maximum at age 30 and then fall [Furuhata & Eguchi (139); Wildführ (140)]. Seifert (141) claims that the antigenic strength of specific organs in man, as a stimulus to the formation of antisera by rabbits, declines after the age of ten years. Although the analytical methods may be questioned, Fumarola & D'Antona (142), as well as Newcomer *et al.* (143), reported a marked fall in properdin in the blood between the ages of 60 and 90. Oberhoffer, Prokop & Schuberth (144) found that subjects over the age of 60 were less capable of producing A-antibody after immunization with Morgan Witebsky blood group protein than those 31 to 40 years of age.

*Radiation.*—In rats, mice, and dogs, exposure to nonlethal doses of radiation shortens the life span. It has been suggested that this increase in mortality is caused by a general acceleration of the "normal aging process" [Alexander (145); Failla & McClement (146); Upton (147)]. Strehler (148) has reviewed the available data and concluded that aging and radiation are unlikely to produce their de-adaptive effects by similar mechanisms because (a) aging is primarily an effect on postmitotic cells whereas, at low dosages, radiation primarily affects dividing cell lines and (b) the lives of some experimental animals are not shortened by large doses of radiation. Comfort (149)

points out that radiation, inbreeding, and aging all diminish the resources of the organism in maintaining homeostasis against random environmental attacks but concludes that further experiments are required before it can be assumed that there is any real identity of mechanisms in the effects of radiation and aging.

Crosfill, Lindop & Rotblat (150) found that in mice the  $LD_{50}$  for deaths within 30 days of irradiation fell from 952 r at 30 to 40 weeks of age to 676 r at 90 weeks. Thus, the older animals were more sensitive to single exposures to radiation than were adults. The long-term effects of radiation in mice showed a similar dependence on age at irradiation [Lindop & Rotblat (151)]. Sacher (152) exposed mice to 100 r of x-radiation per day, 5 days per week, for the duration of life, beginning at ages between 95 and 823 days. In the period from 100 to 600 days of age, the mean accumulated doses at death showed little dependence on age, but, when exposure was initiated at 600 to 800 days, the accumulated dose decreased rapidly.

Although the  $LD_{50}$  fell from 715 r for 6-month-old rats to 600 r for 16-month-old rats [Hursh & Casarett (153)], there was no evidence for an age difference in the rate of repair of residual injury from a single exposure to x-rays.

#### AGE DIFFERENCES IN SPEED OF RESPONSE

Aging is associated with a general slowing in a wide variety of physiological responses. As pointed out in earlier sections, there is a slowing of the circulation and an increased duration of cardiac systole in the elderly. Old subjects require more time to readjust physiological displacements induced by alterations in the environment than young. The age reduction in maximum ventilatory capacity was shown to be caused primarily by reduced ability of old subjects to augment their respiratory rates.

*Reaction time.*—Reaction times become progressively longer with age. The changes are relatively small with very simple tasks but become greater when movements have to be carried out in a continuous co-ordinated series. They are very much greater when complicated rules are introduced which require the subject to relate what he perceives to what he does. Present evidence indicates that most sensorimotor performance in old people is limited by mechanisms of the central nervous system, rather than by impairments of peripheral sense organs [Welford (125)]. Although nerve conduction velocity decreases significantly with age [Norris, Shock & Wagman (154)], the degree of change is not sufficient to be a major factor in the increased reaction times of the elderly. Wayner & Emmers (155) found a significant increase in synaptic delays for the monosynaptic *flexor hallucis longus* reflex in rats between the ages of 117 and 822 days. Although there were no age differences in afferent conduction velocity, ventral root conduction velocity decreased in the old rats. Although the methodology leaves much to be desired, Magladery, Teasdale & Norris (156) have made measurements of reflex and

reaction times in humans that lead them to conclude that the central nervous system plays a primary role in the age-dependent delays in flexion responses.

A number of investigators [Obrist (157); Silverman, Busse & Barnes (158); Busse *et al.* (159); Harvald (160); Friedlander (161)] have reported a slowing of the alpha rhythm of the EEG with advancing age, but the functional significance of the finding is unknown [Magladery (162)].

*Sense organs and perception.*—In addition to the increasing incidence of sensory defects, especially in hearing and vision, there is a systematic rise in threshold values for most perceptual processes [Weiss (163)]. Hinchcliffe (164) claims that sensory losses in both hearing and taste follow an exponential rather than linear regression on age when the threshold stimulus level is expressed by a psychological rather than physical intensity scale. He, therefore, argues that sensory impairments of the aged are partly caused by central nervous system factors and not entirely by end-organ changes. This is an attractive hypothesis that needs further exploration. Said & Weale (165) have designed an ingenious method for determining the spectral transmissivity of the living human crystalline lens by photographic recording of projected Purkinje images at different wavelengths of light. The logarithm of the spectral transmissivity of the lens was constant between 4 and 20 years and then decreased linearly with age up to 63 years. McFarland *et al.* (166) found a systematic increase in visual thresholds under conditions of dark adaptation in 240 males, aged 16 to 89 years. The difference between the thresholds for 20 to 29 and 80 to 89 year olds amounted to 2.5 log units. McFarland *et al.* (167) have also reconfirmed the age reduction in critical flicker frequency and have found that the age decrement increases as the per cent light time in the flicker cycle is reduced.

Taste sensitivity to substances of a sweet, sour, salty, and bitter nature all showed a similar decline for each of the four primary tastes after the age of 60 in 100 subjects, aged 15 to 89, tested by Cooper, Bilash & Zubek (168) using a wide range of concentrations of the test substances. The negative results of Cohen & Gitman (169) and Byrd & Gertman (170) may be ascribed to the use of a few coarse gradations in the concentrations used as stimuli for each of the taste modalities. Bourlière, Cendron & Rapaport (171) tested both perception and recognition for sweet and salty tastes in 81 subjects, aged 20 to 93. Each subject was presented with six concentrations of saccharine and salt (0.1 to 2.0 per cent saccharine; 0.075 to 2.0 per cent salt). For sweet taste, the thresholds of perception and recognition were much higher in subjects 60 to 93 years of age than in subjects 20 to 39 of either sex. For salty taste, only the perceptual threshold was raised with age and then only in men. Lumía (172) tested each primary taste with a series of 22 concentrations and found a decrement in sensitivity that became especially marked after age 70.

Chalke & Dewhurst (173) found that 30 per cent of 61 subjects over age 65 were unable to recognize the odor of "town gas" at concentrations below

50 parts per 10,000. Ninety-five per cent of the subjects under the age of 65 were able to recognize 20 parts per 10,000. Among adults, age does not influence the frequency of reports of pain from dental procedures [Herzberg (174)]. In spite of wide individual differences, Rosenberg & Adams (175) were able to demonstrate an age decrement in vibratory sensitivity which was considerably greater in the big toe than in the fingers. The authors claim that the age regression was logarithmic rather than linear for this variable.

#### AGE DIFFERENCES IN THE EFFECTIVENESS OF CONTROL MECHANISMS

Age changes become more marked as we proceed from cellular metabolism—through the performance of organ systems—to complex behavior of the total man [Shock (176)]. Thus far, the largest age decrements that have been observed in intracellular processes are no greater than 15 per cent in contrast to decrements of 40 to 60 per cent in total organ performances. As pointed out by Gerard (177), with increasing complexity of a system, it becomes more vulnerable to changes in specific sub-units and requires more precise controls to maintain proper functional relationships between its parts. As a working hypothesis, we may assume that impaired performance in the aging adult is related to the breakdown in neural and endocrine regulatory mechanisms.

Regulation of the heart rate is less effective in the old than the young as evidenced by the rising incidence of arrhythmias in the old. The slower response of blood vessels to the vasomotor effects of heat or cold also indicates an impairment of regulatory mechanisms in the aged [de Crinis *et al.* (92)]. Experiments on rats and mice have shown that the mechanisms of heat regulation are less effective in old than in young [Grad & Kral (178); Hügin & Verzář (179)]. Lottenbach & Scharf (180) found that the decrease in pulse rate following a given increment in blood pressure was less in old than in young subjects and attributed the difference to diminished sensitivity of baroreceptors. These results are in accord with the increased thresholds reported in a number of sense modalities. Recovery of the rise in systolic blood pressure following the performance of mental calculations required more time in old than in young subjects [Hurlimann & Imhof (181)].

*Endocrine factors.*—Space will not permit a detailed review of endocrine changes with age, but brief mention of the role of certain specific endocrines as controlling mechanisms will be made.

Although the size of the thyroid gland diminishes with age, there is little evidence for functional impairment of the gland. The basal metabolic rate per  $M^2$  surface area falls with age, but there is no evidence that the rate of cellular metabolism is slower in the tissues remaining in the old person than in the young [Shock (128)]. Other indices of thyroid function, such as protein-bound iodine of the plasma or the 24-hour uptake of  $I^{131}$  by the thyroid gland, are unaffected by age [McGavack & Seegers (182); Wilansky, Newsham & Hoffman (183); Klein (184); Pietra *et al.* (185)]. Furthermore, McGavack & Seegers (182), Zurlo & Grandonico (186), and Baker *et al.* (187) have shown

that the thyroid gland of the aged subject responds to the administration of thyroid-stimulating hormone as effectively as that of the young. Indices of response included increased basic metabolism rate, protein-bound iodine of the plasma, and uptake of  $I^{131}$  by the thyroid. It seems probable that the reduced production of thyroxine by the gland in the old is a physiological adaptation to diminishing requirements and that any deficits that exist must lie in the controlling mechanisms of the anterior pituitary gland or midbrain.

Numerous investigators have shown a progressive fall in the excretion of 17-ketosteroids with advancing age in both men and women [Binet, Baulieu & Jayle (188); Borth, Linder & Riondel (189); Johnsen (190); Schüller (191); Brooksbank & Salokangas (192); Würterle (193)]. Since the steroids measured in the urine represent breakdown products of secretions of both the adrenal cortex and the gonads, the results are difficult to interpret. Migeon *et al.* (194) have observed an age-dependent fall in dehydroepiandrosterone and androsterone in the plasma. However, the inference has been drawn that with aging there is a functional impairment of the adrenal cortex. This inference is open to question, since the eosinophil response to the administration of ACTH is the same in old and young subjects, although the response of urinary excretion of 17-ketosteroids is reduced in the old. Indovina (195) found no age difference in the change in ascorbic acid content of the blood after administration of adrenocorticotropin. Pitzalis, Storelli & Bernardini (196) have reported a slight delay in the development of the eosinopenia that followed surgical operations in elderly people. It appears that the primary source of any decrement in functional capacity of the adrenal cortex lies in the anterior pituitary or midbrain.

Direct estimates of the functional activity of the anterior pituitary in the intact human are not available, because of the lack of adequate methodology. Although the hormone content of the gland can scarcely serve as an index of its functional activity, Bakke & Lawrence (197) observed an average thyroid-stimulating hormone content of  $210 \pm 45.8$   $m\mu$  per gland in 12 patients less than 51 years old and  $82 \pm 10.4$   $m\mu$  per gland in 32 patients over 51. Individual glands obtained at autopsy were assayed by a method utilizing the weight change of surviving bovine thyroid slices. Gershberg (198) assayed saline suspensions of human pituitaries removed at autopsy by observing growth and metabolic effects after injection into hypophysectomized rats. Assays on seven glands from patients ranging in age from 12 to 72 years gave no evidence of age differences in growth hormone content per mg. of gland. Experimental administration of growth hormone to adult rats did not significantly alter their longevity [Everitt (199)].

Using the mouse uterus test, Johnsen (200) has assayed the gonadotrophin activity in 24-hour urine samples in 295 normal males and females between the ages of 25 and 96 years. In men, the excretion of gonadotrophins increased continuously from the age of 30; at age 70, the mean value of this excretion was about twice (58  $m\mu$ ) that in young men (30  $m\mu$ ). In women, the climacteric was associated with a tenfold rise in values (mean, 210  $m\mu$ ).

After the climacteric, there was no further change in excretion of gonadotropins with age in women.

#### AGING AND THE REDUCTION IN RESERVE CAPACITIES

The over-all effect of aging on the individual is a reduction in reserve capacities which reduces his ability to meet stresses of the environment to which he is exposed in daily life. The increasing probability of death with age is a general expression of the loss in reserve capacities. Strehler & Mildvan (201) have reconciled the linear decrements in physiological functions with the logarithmic increase in the probability of death by assuming that the magnitudes of challenges (or the responses required to overcome them) are distributed energetically like a Maxwell-Boltzman distribution of energies among molecules ( $n/n_t = K(E/RT)^{1/2}e^{-E/RT}$ ). When this equation is combined with the Gompertz equation describing mortality ( $R = A + R_0e^{at}$ ) and applied to human mortality data, a number of significant predictions can be made; namely, (a) loss of function with age is linear with time, (b) the calculated percentage loss in physiological function per year ranges from 0.9 to 1.4 per cent per year, (c) there is an inverse linear relationship between the slope of the Gompertz function and its intercept, and (d) the mean ratio of maximum reserve capacity to average demand for physiological functions lies between 7 and 11. These predictions are roughly in accord with observational data.

The reduction in performance or reserve capacities in an organ system may be caused either by a gradual loss of functional units or by impairments of the remaining units, or a combination of both.

*Anthropometric measurements.*—A number of anthropometric measurements lend support to the hypothesis that at advanced age there is a loss in body tissue. Master, Lasser & Beckman (202) found a significant decrease in average weight of successive 5-year age groups in a population of 2925 males and 2694 females, between the ages of 65 and 94. Of course, part of the decrease in average values may be caused by progressive reduction in the proportion of overweight subjects because of the relatively higher mortality rate of obese as compared with underweight individuals. Until serial determinations of body weight of single individuals are available, it is impossible to determine how much of the loss in weight is a loss of body substance. However, it may be pointed out that a terminal loss in body weight is also present in aging rats [Everitt (203); Everitt & Webb (204)].

Lee & Lasker (205) and Cowan (206) were unable to detect any significant regression of body weight with age among patients in an old people's home or those presenting themselves at a consultation health center for old people. This is not surprising since body weights are, on the average, significantly lower in these groups than in the general population. Cowan (206) did find a significant fall in body weight of women. Lee & Lasker (205) were also unable to detect any significant change in subcutaneous fat as estimated by spring-loaded skin fold calipers.

*Body composition.*—The total body water content, determined by the antipyrin dilution technique, diminishes significantly with age [Shock, Watkin & Yiengst (207); Olbrich, Woodford-Williams & Attwood (208); Parker *et al.* (209); Ruol *et al.* (210)]. The extracellular water, estimated by the thiocyanate dilution technique, does not change with age but constitutes a larger proportion of the total body water in the old than in the young. Since the intracellular water concentration does not change with age, it may be inferred that the reduction in total body water is a reflection of tissue loss at advanced ages. Anderson & Langham (211) counted gamma rays from natural  $K^{40}$ , with a  $4\pi$  liquid scintillation gamma counter in 1590 males and females, aged 1 to 79 years. After the age of 20, there was a linear decrease in potassium concentration (gm. per kg body wt.) in both sexes, which amounted to about 18 per cent in males and 6 per cent in females. The authors believe that their results indicate a loss in lean protoplasmic mass. Sagild (212) and Macgillivray *et al.* (213) have observed decrement, with age, in total exchangeable potassium in normal subjects.

*Tissue composition.*—Histological studies uniformly show an increase in connective tissue in most organs with advancing age. Many studies of connective tissue from a wide range of organs (lung, skin, blood vessels, and tendons) show that age brings about significant changes in the physical and chemical properties of connective tissue. Space does not permit a detailed review of these findings, but the following references may be cited: Verzár (214, 215); Kohn & Rollerson (216 to 218); Kaplan & Meyer (219); Chvapil & Hruza (220); Banga (221). In brief, collagen from old animals is characterized by (a) greater insolubility toward chemical agents and collagenases, (b) alterations in its contractility when exposed to chemical agents or temperature changes and (c) increased rigidity of the collagen fibril. These changes in physical properties of collagen play an important role in the age changes already described in blood vessels, lungs, and perhaps other tissues, such as muscle and skin, as well.

The hypothesis has been advanced that aging produces greater rigidity in the collagen molecule, because of increased chemical cross-linking between spiral strands of the molecule and between the collagen fibrils themselves. Although this hypothesis is reasonable and attractive, much additional experimental work is required before it can be accepted.

The close relationship between age decrements in glomerular and tubular functions of the kidney leads to the presumption that nephrons are lost in the old kidney. Histological studies lend support to this presumption, although the data are scanty. Direct histological evidence for loss of cells from specific organs and tissues with advancing age is contradictory. Birren & Wall (222) were unable to detect any differences in the number of fibers in the sciatic nerve of rats between the ages of 50 to 850 days. Similar negative results were reported by Moyer & Kaliszewski (223) in their study of fibers in motor spinal nerve roots of 16 cats aged 10 to 18 years. These results stand in contrast to previous reports in the literature on total nerve cell counts in



man summarized by Wright & Spink (224). These studies show a 20 to 30 per cent reduction in the number of nerve cells in olfactory glomeruli, optic nerves, Purkinje formation, and in the cerebral cortex. Wright & Spink (224) found a drop of 15 to 20 per cent in nerve cell counts in the spinal cord of mice aged 110 weeks.

The number of mast cells in the vascular wall diminishes with age in man [Sundberg (225)]. Constantinides & Rutherford (226) found a diminution in the number of mast cells in the myocardium of rats by the age of 18 months.

Andrew *et al.* (227) reported a significant loss of protoplasmic units from muscle tissue in old rats (24 months of age). Histologic evidence agreed with biochemical data on the same tissue, which showed an increase in the proportion of extracellular to total water and a reduction of potassium in the old muscle tissue.

It seems probable that tissue loss plays some role in aging. However, it must not be assumed that this is the only, or even the primary, basis of aging. There is a substantial body of literature, which cannot be reviewed here, that indicates alterations in specific cellular processes with aging. Furthermore, it seems highly probable that changes in cellular function must precede their death and disappearance from a tissue or an organ. In addition, we know that mortality rates in many organisms other than mammals follow the same general progression. Further research, with more precise and sophisticated methods, is required to understand the cellular mechanisms involved in aging.

It is apparent that much of the data on age changes is still descriptive in nature. Even at this level, there are many gaps in our knowledge so that further descriptive studies on large, well-defined populations are necessary. However, the future of gerontology depends on the design of experiments to investigate the mechanisms of age changes.<sup>5</sup>

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# RESPIRATION<sup>1</sup>

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During the past year there have appeared a number of review articles or monographs either in the field of respiratory physiology, or sufficiently closely related to be of interest to the respiratory physiologist. Volume 2 of the new *Handbook of Physiology* contains a chapter on the neural control of respiration by Oberholzer & Tofani (204). Bouhuys & Lundin have reviewed the general problem of the distribution of inspired gas in the lungs (33). An atlas of the submicroscopic structure of the lung in both health and disease appeared (244) that provides many fascinating details for the physiologist concerned with the structural determinants of function. Two lucid, comprehensive analyses of the kinetics of reactions between hemoglobin and gases (115) and on diffusion and simultaneous chemical reaction velocity in hemoglobin solutions and red cell suspensions (232) appeared. The circulatory adaptation to hypoxia (154), the physiology and pharmacology of the pulmonary circulation (181), and the principles of oximetry (199) were the subject of extensive reviews. Ferris (103, 104) has assembled much of the current literature on pulmonary function. Finally, special mention must be made of a scholarly essay by Richards on homeostasis (223), not directly concerned with respiration, but of interest to students of the arrangements and derangements of function in any system.

## MECHANICS OF RESPIRATION

Understanding the intimate nature of the performance of the ventilatory apparatus as a mechanical system continues to be a challenge to the respiratory physiologist. By sheer weight of number of publications under the generalized category "mechanics", this area of research represents the most actively studied segment of respiration. Fenn has written a lucid summary of the mechanism of breathing (100), simply presented but profound, and singularly well suited to serve as a teaching reference in physiology.

The fundamental nature of the contribution of various tissues of the lung to its "elastic" performance is poorly understood, but an appreciation of the importance of surface tension effects has been a particularly noteworthy advance (43). There is apparently on the alveolar surface a peculiar material which has a coefficient of compressibility that places it in the category of a liquid film. The surface tension of this material decreases markedly on compression. The process is reversible if it is compressed to about 50 per cent of its surface area, but with further compression the film appears to rupture on re-expansion. This behavior is at variance with that seen in pure protein films, and although it has not been possible to draw a firm conclusion, the

<sup>1</sup> This review covers the literature available to the author for the period ending June 1, 1960.

presumptive evidence suggests that the important material in the film is a mucoprotein.

The correlation between pressure-volume data of the lung and force-area relationships of a mucus surface allows deductions concerning the surface tension effect from pressure-volume data of the lung as a whole, although at small lung volumes the closure of units and, therefore, change of effective surface area introduce an error. Calculation of lung surface area from these measurements provides a value of 70 m.<sup>2</sup> in man, a figure somewhat higher than previously estimated. In three different species with widely varying body weights and lung sizes, the computed lung surface area at functional residual capacity appears to correlate linearly with the body weight on a log-log plot.

*Resistance and compliance.*—With the passage of time, the compliances of a surface film extracted from the lungs, of isolated lungs, and of lungs in an intact animal all decrease. In studying this phenomenon in lungs of anesthetized dogs, Mead & Collier (183) found that the fall in compliance could be reversed by intermittent re-inflations and pointed out that the final functional effect on gas exchange of the lung will be different if the compliance change, on the one hand, is due to a diffuse effect of surface film aging or, on the other, to the closure of discrete alveolar units. Post-mortem observation demonstrated air-space closure, and by employing two techniques for the measurement of lung volume, one dependent on airway patency and the other independent of airway, it was determined that the closure was of the atelectatic type. The contribution of vascular changes to compliance change in these studies was thought to be small. The pulmonary compliance in unanesthetized man too has been shown to decrease after a deep breath (101), a particularly dramatic observation if the deep breath follows a period of chest strapping (59). In the latter instance there was also a rise in the end tidal nitrogen concentration, with the deep breath suggesting a sudden opening of previously nonventilated terminal lung units. Whatever the mechanism, it is clear that an appreciation of the previous "volume-history" of the lungs is extremely important when comparing normal values or examining the effect of some experimental variable.

Pulmonary resistance decreases when the lungs are in a state of inflation (52, 62, 249) probably because of increase in cross-sectional area of the flaccid airways, although it is known that minor changes in airway pressure can increase the diameter of bronchi and even the trachea (147). Length changes were of less importance in this study, but Harris (128) studied the extensibility of the isolated human trachea and concluded that free tracheal extension is important in the mechanism of respiration, as it facilitates the inspiratory descent of the lung root. Butler *et al.* (52) noted that even at a constant lung volume, airway conductance increased when lung elastic pressure was increased after chest constriction. This would indicate that lung airway conductance varies directly with lung elastic pressure, and its relationship to

lung volume is determined by the pressure-volume behavior of the lung.

The interrelationships of the pulmonary blood volume and the mechanical characteristics of the lung continue to be a significant problem. For many years it had been noted that isolated lungs swell when injected with blood, without any significant displacement of air from alveolar spaces; but in man an increase in pulmonary blood volume was usually associated with a fall in vital capacity and total pulmonary capacity, apparently the result of a displacement of air space by the increment in blood volume. Now Frank has shown in excised cat lungs (109) that pulmonary congestion results in a reduction of slope of the volume-pressure relation of the lungs at all volumes, but that the recoiling force of the lungs is a function of the volume of the lungs at which congestion is produced. With large lung volumes, congestion increased the recoiling force, but with small lung volumes, it was reduced. There was an intermediate volume close to that at which tidal breathing occurs where congestion produced no change in recoiling force. In addition to the structural-mechanical factor brought about by the proximity of blood vessel and alveolar wall in the lung, there is also a reflex mechanism, stimulated by a fall in systemic blood pressure that leads to a rise in tracheal pressure at constant tidal volume (166). The effect is abolished after vagotomy, although the sites of receptors in this reflex arc are not known, nor is it even clear whether the phenomenon is the result of airway resistance or pulmonary compliance. Vagal stimulation alone results in a decrease in volume of the isolated lung (27), and if prolonged, this decrease becomes irreversible.

A comparative approach to the mechanics of breathing in which dogs, cats, rabbits, guinea pigs, and rats were studied indicated that lung-thorax compliance per unit body weight and per unit vital capacity showed significant differences between species (2). The static pressure for a normal inspiration, and the dynamic pressure required for a normal minute volume, were similar in all animals. The important generalization suggested is that the ratio of expiratory reserve volume to vital capacity, or of functional residual capacity to total capacity, is larger in those animals that breathe at a lower respiratory frequency. This volume change would tend to stabilize the alveolar gas concentrations during the breathing cycle, in spite of a low respiratory frequency. Furthermore, the normally selected respiratory frequency provided the minimal value for respiratory work throughout the several species. A separate study comparing the mechanical properties of the dog and pig lung showed that the pig lung is less compliant than that of dog or man, but the ratio of compliance to lung weight brings pig and man to about the same value (13).

In the newborn animal, the compliance of the lungs and thorax, per unit of vital capacity, is larger than in the adult when examined over an extended range, but it is smaller over the tidal volume range (3), probably because many nonaerated alveoli are present. After a few days of life, the compliance of the lungs is similar to that of the adult, but the compliance of the chest

wall, particularly high at birth, progressively declines. After death the effect of degree of lung inflation on the pressure-volume relationship becomes much exaggerated, probably because there is a tendency for airways to collapse (249).

Of the various inhaled substances that affect respiration, gaseous ions are of particular interest. Positive air ions depress ciliary activity and contract the tracheal wall; negative ions accelerate ciliary beat and will counteract the effect of positive ions on the tracheal wall (156). Indirect evidence indicated that positive ions release 5-hydroxytryptamine while effects of negative ions depend on their ability to accelerate the enzymatic oxidation of 5-hydroxytryptamine (157). Inhaled dusts produced a decrease of compliance, increase of airway resistance, and increase of the volume of thoracic gas (18, 69, 123). Both histamine and epinephrine increased the elastic resistance of the lung (44).

A number of earlier reports indicated that pulmonary compliance was greater in the upright than in the supine posture, an observation which has been extended to exercise in these two positions (118, 212). As in most lung studies in man, the esophageal balloon was used as an index of intrathoracic pressure. The extent of difference between intrathoracic and esophageal pressure depends on the elastance of the esophageal wall and the pressure-volume coefficient of the measuring equipment, but as a rule the technique is entirely valid (187). In the supine position, however, the mediastinal contents compress the esophagus and give a falsely high value for intrathoracic pressure (102, 151). When a correction is made for this volume artifact, static and dynamic compliance values show no significant difference between the upright and supine positions.

Knowledge concerning air flow in the various components of the respiratory tree has been based largely on calculations from data of volume flow and regional cross-sectional area. The significance of local anatomical peculiarities to the pattern of air flow is largely conjectural, but the importance of precise knowledge concerning flow rates at which laminar is converted to turbulent flow, and the regions in which this occurs, is acknowledged as a necessary background to the appreciation of alveolar ventilation and gas exchange under many different circumstances. West & Hugh-Jones (271) studied this problem in models reconstructed from lung airway casts and also in intact dogs. They found flow to be laminar throughout the tracheobronchial tree at low flow rates (10 l./min.) but turbulent proximal to the segmental bronchi at high flow rates (80 l./min.). At both high and low flow rates there was incomplete mixing in the main bronchi, an observation of great significance to the technical problem of sampling. The irregularities of the tracheobronchial tree made an estimate of a critical Reynold's number difficult, particularly in the main and lobar bronchi. Although steady flow only was used in the study, the authors mention that with cyclic flow it may take a long time to re-establish laminar flow after turbulence and that this may be important at the end of a normal respiratory cycle when flow is low but turbulence may per-

sist. In emphysematous subjects helium is without effect on compliance but reduces resistance to breathing by about 20 per cent (119).

*Work of breathing.*—The simplest mathematical assumption in treating the respiratory cycle is that a sine wave is a reasonable approximation. Measurements of the rate of external work in eight subjects breathing voluntarily, then ventilated at the same minute volume by a sine-wave pump, indicated values that were about equal (70). The nose constitutes a rarely mentioned resistance to breathing. Butler (51) found that there is a wide variation in nasal resistance, but that in general it is about one and one-half times that of the lower airway resistance; and when breathing at a rate of 20 breaths per min. and with a 500 cc. tidal volume, the work of lung movement is doubled when breathing through the nose compared to through the mouth.

A newer estimate of the efficiency of the chest wall and diaphragm in ventilation is that it is less than 7 per cent in normal subjects (111). Although this value is not much different in cases of pulmonary emphysema, obese patients have an efficiency lower than normal, apparently because of the extra work load imposed by moving the adipose tissue of the chest wall and abdomen. Pregnant women consume more oxygen per liter of ventilation (17) than do normal nonpregnant subjects, and this too may be caused by the extra load imposed on the diaphragm, or it is possibly related to the altered relationship between thoracic and diaphragmatic respiration.

*Lung volumes.*—Except for diagnostic purposes the lung volumes are no longer the subject of much investigation in laboratories of respiratory physiology. Singers have lung volumes no different from those without voice training (130), but trained athletes have greater vital capacities than nonathletes (256), though the maximum breathing capacity showed no difference between the two groups.

The change in the expiratory reserve volume between sitting and supine positions was re-examined by Craig (74). By studying the effect of accessory maneuvers such as supporting the elbows on the arms of a chair, or leaning forward in the sitting position, the importance of the weight of the shoulder girdle and abdominal contents in determining the final volume was emphasized. Transverse acceleration results in a restrictive effect on the chest and limits primarily the vital capacity (63).

The respiratory dead space is important for the role it plays in detracting from the primary functional role of the lung. Emphasized by many as a "space" not a volume, intuitively clear but conceptually obscure, it still rates in most people's minds the assignment of a numerical value. The single-breath technique for the measurement of the "anatomical" dead space continues to provide useful information. With this method, the dead space was found to be independent of the washout time in both model systems and human lung; but the dead space, so measured, decreased in human subjects if there was an inspiratory pause, while in model systems this maneuver was without effect (29). While the dead space volume depends on the tidal volume (29) or, probably more accurately, on the functional residual capacity, its de-

pendence on expiratory flow velocity remains unsettled. Herberg *et al.* (131) found a 10 cm.<sup>3</sup> increase in "absolute" dead space for each 100 cm.<sup>3</sup> per sec. increment in expiratory flow velocity.

Examination of the giraffe lungs and trachea revealed an anatomical dead space of about 1600 ml. (225).

#### CIRCULATION OF THE LUNG

*Pulmonary circulation.*—The physical characteristics of the pulmonary vascular bed important to a complete description of the factors regulating pulmonary blood flow are in many ways unique. Most authors have measured the pressure drop across the vascular bed at various rates of flow, but few have stressed the importance of the vascular transmural pressure gradient. Resistance to flow, insofar as it is determined by the number of open channels available, depends on the stability of the vessels, and this in turn is a function of wall tension and transmural pressure. In the lung the pressure outside the vessels is usually the alveolar pressure, and throughout the lung this value may not be uniform. Lloyd & Wright (173) perfused isolated, submerged, gas-free, and fluid-distended dog lungs to study the vascular resistance as a function of transmural pressure with a technique that abolished gravitational effects. Their results indicated that the pulmonary vascular bed behaves as a compliant tube of limited maximal radius to which it may be distended while still in the normal pressure range. In no instance did they observe cessation of arterial flow indicating a critical closing pressure, although others find that closure may occur when the pressure falls below 7 to 8 mm. Hg (234). In gas-filled lungs, blood flow persisted even when bronchial pressure exceeded perfusion pressure, but this was no longer the case when fluid began to collect in the alveolar spaces. This observation indicates that part of the effective transmural gradient is determined by surface tension forces. With a somewhat different approach, Cross *et al.* (75) found that regardless of the presence or absence of liquid in the bronchi, vascular pressure was determined by airway pressure. The usual formula for calculating pulmonary vascular resistance assesses only resistance to flow and may be misleading in deductions of vascular tone (234).

Sudden collapse of the lung causes a reduction of right ventricular output and fall in pulmonary artery transmural pressure (21) which return towards normal in the steady state. Local atelectasis causes first a fall, then a rise in vascular resistance (15). The former is largely a mechanical effect, but the latter involves a local effect of CO<sub>2</sub> on the pulmonary veins or capillaries. The very large change in blood flow through a chronically atelectatic lobe cannot be accounted for by any of the mechanisms seen in acute atelectasis and probably involves chronic organic changes. The local vasoconstrictor effect of CO<sub>2</sub> was also observed in the mechanically ventilated and perfused left lower lobe of the dog's lung but in this case, by contrast with the study in acute atelectasis, the locus of action was presumed to be on the arterioles (179). The most important determinant in this case seemed to be the pulmonary arterial-pulmonary venous CO<sub>2</sub> tension gradient. With either high or

low  $\text{CO}_2$  tensions in the pulmonary artery, pulmonary vein, or alveoli, no significant hemodynamic effect was seen if the gradient remained large. A decrease in alveolar and pulmonary venous oxygen tension caused a rise in local pulmonary vascular resistance (179), but in the case of atelectasis (15) any vasoconstrictor effect was mediated via carotid and aortic chemoreceptor activation and was not a local response. Yet unilateral hypoxia in man results in a selective shunting of blood away from the hypoxic lung (84). This much-studied problem of the action of oxygen and carbon dioxide on pulmonary blood flow and vascular resistance is still controversial. Different authors, using different preparations and different species, report conflicting results and, quite naturally, offer widely differing interpretations.

In a whole-animal preparation in which the vasosensory areas of the carotid bifurcations and of the aortic arch, the brain, the remainder of the systemic circulation, and the lungs were separately perfused, a study was made of the effects of chemoreceptor stimulation on the pulmonary vascular bed (80). The preparation provides a great deal of control over the various secondary reflex and hormonal effects that have led to so much confusion in the study of actions of oxygen and carbon dioxide referred to above. Hypoxic stimulation of the carotid body chemoreceptors produced an increase in pulmonary vascular resistance regularly only if bronchial blood flow was interrupted. If bronchial flow was maintained, the pulmonary arterial effect was often masked by a passive mechanism altering the distribution of blood between the bronchial and pulmonary vascular beds. Carotid baroreceptor stimulation caused a reflex dilation of the pulmonary vascular bed arising from inhibition of sympathetic vasoconstrictor tone (79).

The bronchial circulation in dog and man has been measured by a dye technique (76) and indirectly by the simultaneous measurement of left and right ventricular output, it being assumed that left ventricular output will exceed right ventricular output by the amount of bronchial blood entering the pulmonary veins (77). By this latter method the bronchial blood flow was estimated to be about 67 ml./min. If one pulmonary artery was ligated, the difference between left and right ventricular outputs far exceeded the normal value, signifying an enlarged bronchial system. Shunting of pulmonary arteriovenous blood in both normal subjects and patients with heart disease has been demonstrated by following the arterial oxygen saturation during, and immediately following, a Valsalva maneuver (144).

Hypertonic saline caused a rise in pulmonary arterial pressure which was thought to be secondary to a mechanical blockage of the small vessels caused by an increased tendency for the red cells to agglutinate (246). Isoproterenol in large doses is reported to cause pulmonary arteriolar vasoconstriction and, in smaller doses, pulmonary vasodilation (215), but the failure to assess quantitatively the role of pulmonary blood flow in these instances leaves the interpretation of these pressure changes subject to doubt. Serotonin is a potent pulmonary vasoconstrictor, but some of the accessory factors that must be appreciated in analyzing its effect were emphasized by Rudolph & Auld (234) who point out that an increase in pulmonary blood flow will, of itself,



decrease pulmonary vascular resistance and may reduce the pressure to normal even though serotonin has acted to increase the vascular tonus. Aviado considers that the primary site of action of serotonin is on the pulmonary venules (14).

*Pulmonary blood volume.*—Techniques for measuring the amount of blood in the lungs are of necessity indirect, but in addition, the associated assumptions and complexities of measurement call for special caution in exact interpretation. The "slope-volume" analysis of indicator dilution curves, when compared with the Stewart-Hamilton method, was found to provide a value only 30 per cent of the volume in the lungs and left side of the heart and probably about 45 per cent of the pulmonary blood volume (265). Even as an index of the magnitude of change in pulmonary blood volume induced by temporary occlusion of the pulmonary artery, the "slope-volume" method was found to be seriously wanting. The indicator dilution method, when applied to a variety of sampling sites in dogs, provided data for the calculation of the various volumes that together comprise the "central blood volume" (180). The mean volume between the femoral vein and femoral artery was 358 ml./10 kg. of body weight, of which a third or less was in the lungs. The mean volumes for the various components (ml./10 kg.) were venous, 98; right side of heart, 44; lungs and left side of heart, 149; arterial, 80. If peripheral sites were used to sample indicator dilution curves, few deductions could be made about the actual, or even relative, volume of blood in the lungs (180). The Stewart-Hamilton method too contains a measurable error ascribable to the nature of mixing characteristics of the vascular system (209).

Measurement of radiation from a circumscribed portion of the lung following the injection of radio-iodinated serum albumin (171, 266) does not provide a quantitative measure of pulmonary blood volume, but it is satisfactory for assessing relative change during an experiment. It has allowed the graphing of relative pressure-volume characteristics of the pulmonary vascular bed (171) and relative change in pulmonary volume on change of posture (266). The pulmonary blood volume decreases 28% in the upright posture and 11 per cent after a Valsalva maneuver.

#### CONTROL OF RESPIRATION

*Central nervous system.*—The rhythmic nature of the respiratory center has been explained by two fundamentally different mechanisms. One holds that there is a spontaneous or automatic process in the respiratory center neurons analogous to that occurring in pacemaker regions of the heart. The other mechanism explains the rhythmicity as the result of a reciprocal innervation of the inspiratory and expiratory neurons in the brainstem. In a detailed study exploring these two hypotheses (48, 237, 238), no evidence was found supporting the existence of respiratory pacemaker cells. Progressive neurological isolation of the brainstem reduced the number of periodically active cells in the medullary respiratory area, establishing that the activity is manifest only in an environment of active neurons outside the respiratory group. Although it might be that these extrarespiratory-center neurons serve

to facilitate the cells of the respiratory centers which are themselves spontaneously active, the experimental evidence did not support this possibility. The discharge of respiratory neurons was found to be fundamentally irregular, and dependent upon the integrity of the interconnected inspiratory and expiratory systems, each of which acts by a process of self-excitation. The connections between these two systems enforce a reciprocal innervation such that when there is greater activity in one net there will be a reduction in the other. The "spill over" of inspiratory activity in the intercostal muscle activity during expiration (120) illustrates the temporal lag at the periphery in this complex interaction. Expiratory muscle activity may not even begin until most of the tidal air has been exhaled (106), and with full relaxation and quiet breathing this activity is restricted to local regions (258).

The level of excitability of the respiratory centers is enhanced by  $\text{CO}_2$  as evidenced by both the frequency of discharge and the total number of functional units (48), a conclusion supported by an electromyographic technique as well (219). A preparation with partial cord transection at  $\text{C}_2$  leaving intact only the anterior funiculi has provided evidence for an important integration of respiration at cord level (218) possibly related to the respiratory potential fluctuations (164). In the other extreme, the cortical control of respiration was emphasized by reinforcing conditioned respiratory response with  $\text{CO}_2$  (116) and by establishing conditioned respiratory reflexes of the second order in cats (262).

The respiratory centers in the albino rat are organized in a manner similar to those of other species (207), but there is some evidence that expiration is a more active process.

*Afferent stimuli.*—Pressoreceptors, the stimulation of which modify respiratory activity, have been identified in the pancreatic duct (46), portal circulation (158), and accessory nasal sinuses (198). In animals with an artificial pneumothorax, inspirations begin at specific time phases of cardiac activity (45), and repetitive stimulation of the large fiber components in afferent trunks from tendon and muscle enhances ventilation (26).

The most important group of stretch receptors serving to regulate breathing is located in the lung. The initial apnea that attends radial acceleration when the force is in the head-to-tail direction can be prevented by abdominal counter pressure or bilateral vagotomy, indicating that the apnea is the result of inhibitory action from stimulation of pulmonary stretch receptors (19). Transverse acceleration results in a slight increase in minute volume (63). The cessation of respiratory effort after tracheal occlusion in birds is less dramatic than in mammals but is sensitive to the lung volume at the time of occlusion because of the number of afferent impulses travelling over the vagus (31). Mithoefer (189) carefully analyzed the effect of lung volume on the breath-holding stimulus. The earlier literature suggested that the lung volume served as an independent stimulus for breath holding when the breaking point was correlated with the  $\text{CO}_2$  tension, but the correlation with oxygen tension was poor. Mithoefer's study showed that in order to demonstrate the correlation with hypoxic stimulus and to establish the lung volume

as an independent stimulus it is necessary to correct for the time lag caused by circulation time to the respiratory center. At small lung volumes the hypoxic stimulus is easily masked because of the rapid change in oxygen tension. It is the final volume of the lungs that is important to breaking stimulus, not the initial volume or the rate of shrinkage.

*Carbon dioxide and hydrogen ion.*—The difficult problem of separating the  $\text{CO}_2$  stimulus from the change in pH that it brings about has been re-investigated by the "iso- $\text{P}_{\text{CO}_2}$ " technique (89). By monitoring alveolar  $\text{CO}_2$  continuously the  $\text{CO}_2$  concentration in the inspired air could be adjusted to keep the alveolar value relatively constant during the infusion of 0.5 M HCl, and in other experiments the arterial pH was kept constant during  $\text{CO}_2$  administration. Carbon dioxide and hydrogen ion emerged as separate and additive stimuli, but blood and respiratory center cellular changes may not have been identical. The sensitivity to  $\text{CO}_2$  was enhanced in the presence of metabolic acidosis, but the preparations under study were probably moderately depressed because of barbiturate anesthesia (4). In man, metabolic acidosis produced by  $\text{NH}_4\text{Cl}$ ,  $\text{CaCl}_2$ , and acetazolamide was not attended by any increase in ventilatory responsiveness to  $\text{CO}_2$  (165). The  $\text{CO}_2$  stimulus—ventilatory response curves were shifted symmetrically to the left indicating that at all levels of ventilation a higher ventilatory value for a lower  $\text{CO}_2$  tension. In unanesthetized man (174), independent  $\text{CO}_2$  and hydrogen-ion effects on ventilation were confirmed.

Two more studies in man again raised the question whether respiratory response is not more accurately assessed by a measure of incremental mechanical work rather than the more generally used measurement of minute volume of ventilation (41, 96). With a constantly maintained stimulus, added airway resistance results in a decreasing ventilatory response in normal subjects. This observation is commonly extrapolated to interpret the commonly observed decreased ventilatory response reported in patients with obstructive emphysema, but it was pointed out (41) that in this group the ventilatory mechanical work output in response to a  $\text{CO}_2$  stimulus is also decreased by comparison with normal man. Others have assumed that the response of the respiratory center and the work output of the respiratory muscles in patients with emphysema were essentially normal, but the crux may be a decrease in efficiency of the muscles in emphysema, a fact observed by some (41) but not by others (111).

A new investigation of the  $\text{CO}_2$  stimulus to respiration by the method of measuring the duration of apnea and the alveolar  $\text{CO}_2$  tension at time of the first breath after a period of voluntary hyperventilation with various inspired  $\text{CO}_2$  mixtures concludes that factors other than hypocapnia are of importance (186). Klocke & Rahn (148) studied breath-holding for periods up to 14 minutes after hyperventilation with oxygen with the result that the lung volume shrank by an amount equal to the vital capacity. In this case there is little doubt that neurogenic impulses originating from lung receptors will come into play to summate with the chemical stimuli.

It is widely appreciated that the ventilatory response to  $\text{CO}_2$  varies, depending on whether it is endogenously produced by the body's metabolic activity or whether it is administered in the inspired air. Yamamoto (282) presented a mathematical analysis of this situation and showed that when  $\text{CO}_2$  enters via the blood, increased  $\text{CO}_2$  is eliminated while mean  $\text{CO}_2$  is kept constant but with increasing fluctuations about the mean. In the case of  $\text{CO}_2$  entry via the lung, increasing  $\text{CO}_2$  is eliminated by raising the mean, but at the same time oscillations decrease.

*Hypoxia.*—The immediate response to 10 per cent oxygen in unanesthetized rabbits illustrated that ventilatory effects appear with mild degrees of hypoxia without any concomitant cardiovascular manifestations (153). The elimination of the cyanide gasp by breathing oxygen, first demonstrated by von Euler in cats, was studied further in man (71). The nature of the cyanide-oxygen interrelationship at receptor site is poorly understood, but these authors found that enrichment of oxygen in the inspired air to as little as 30 per cent is sufficient to block the gasp reaction.

*Other chemical stimuli.*—A wide variety of chemical agents affect respiratory activity, some through peripheral mechanisms, some central, and some probably by reactions that secondarily alter respiration, for example, through changes in metabolism. Ammonium chloride first stimulates, then depresses, respiratory activity when perfused through the fourth ventricle (176); these responses are independent of the pH of the perfusate. Chlorpromazine (243) inhibits respiration by stimulating some chemoreceptor area in the chest and can be blocked by severing the vagus. Epinephrine (159) stimulates respiration, probably via action in the central receptor area, and may be of considerable functional importance in the regulation of ventilation during muscular exercise. Progesterone (78), particularly after administration for several days, causes some increase in ventilation in patients with pulmonary emphysema, but the mechanism is far from clear and the data are neither consistent nor conclusive. Serotonin stimulates respiration, apparently by acting through a receptor site on the arterial side of the systemic circulation (208). Natural sleep, the hypersomnolence of narcolepsy, and drug-induced sleep (22, 30, 252) are all attended by respiratory depression manifested by a rise in  $\text{CO}_2$  tension and slight fall in arterial oxygen saturation. Ventilatory response to  $\text{CO}_2$  is decreased, probably because of a depression in central neuronal activity, but it is likely that this may in part be a manifestation of a decrease in the number of afferent stimuli impinging on the central mechanism, by comparison with the wakeful state.

The effects of a change in body temperature on respiration are complicated by the many physiological and physical factors concerned. Severinghaus (247) reviewed the way in which changes in gas solubility and changes in the lung very nearly balance the decrease in metabolism brought about by hypothermia. In addition to the central effect which results in respiratory depression, there is also a more pronounced respiratory inhibition elicited by stimulation of the central end of the cut vagus during hypothermia than

occurs at normal body temperature (150). An interesting observation directly pertinent to the development of respiratory control in newborn rabbits has been reported by Adamson (1). In a neutral environment, hypoxia causes no change in oxygen consumption, but a pronounced hyperpnea. A cool environment caused an increase in oxygen consumption and moderate hyperpnea, but the administration of a low oxygen mixture caused a decrease in oxygen consumption and little or no change in breathing. The metabolic effect is not related to shivering alone, and it is perfectly clear that the ventilatory response is not entirely due to a depression of the respiratory center as previously supposed.

High barometric pressure influences respiration in man and has important connotations to both chemical and reflex control (133). Eight subjects were studied at pressures up to 4 atm. breathing 100 per cent oxygen, 5 per cent oxygen in nitrogen, and atmospheric air. The increase in gas density at high pressures was causal in decreasing the respiratory frequency and in increasing the tidal volume, but the increased partial pressure of oxygen was also considered to result in a decreased chemoreflex drive. At 4 atm. pressure the oxygen was slightly stimulating, its effect overriding the depressant effect of increased resistance, and alveolar ventilation increased. No depressant action of nitrogen appeared at any oxygen tension studied.

#### HYPOXIA AND HIGH ALTITUDE

Acclimatization to chronic hypoxic stress involves many organ systems and cellular and chemical mechanisms. Short-term exposure of rats to a reduced barometric pressure indicated a decrease in oxygen affinity (97) that was probably related to the decrease in blood pH caused by the high blood lactate in the animals. With an exposure of two months at 20,000 ft. there was some increase in muscle myoglobin, but the equilibrium constant had the same value as in the control group (255). The well-known rise in blood hemoglobin concentration is apparently the more important adaptive response in the mature animal, but it is interesting that in the developing chick exposed to different environmental oxygen tensions, the hemoglobin concentration in all groups was the same (9). However, in these developing animals the area vasculosa reacted to lack of oxygen by increasing its size. For some reason, increase in blood fat level results in a fall of arterial oxygen saturation which is more pronounced at high altitude (257). Persons with a defect in fat tolerance may have a decreased ability to withstand oxygen lack. By contrast, food-deprived animals had an increased tolerance to hypoxic stress (196) as did irradiated rabbits, but the radiation effect was not seen in the other species studied (mice, guinea pigs, and hamsters). In adult man, basal oxygen consumption does not change at high altitude (16), but in guinea pigs exposure to 8 per cent oxygen resulted in a fall of body temperature and decreased oxygen uptake (213, 214). Adult animals are known to withstand hypoxic conditions less well than infants, and this has been confirmed in new studies on dogs, rabbits, kittens (60), and rats (85). While in the adult, respiratory failure usually follows circulatory failure in hypoxia, in the infant the reverse

order of failure is usual. The circulatory and respiratory adjustments to hypoxia and muscular exercise have been contrasted (217), but the authors' conclusion that this difference is explained by a difference in the amount of blood in contact with alveolar air is not obvious.

#### OXYGEN AND CARBON DIOXIDE

High concentrations of these two gases evoke a variety of physiological responses many of which are not strictly respiratory but are of great interest to the respiratory physiologist. In rats, one atmosphere of oxygen as a substitute for normal inspired air resulted in a slight increase in resting oxygen consumption (112), an effect opposite to that reported in man and by inference in rats (in which case  $\text{CO}_2$  output was measured) (283). The observed effect seemed to depend on environmental temperature and, for this and other reasons, was attributed to a true effect on metabolism, not an artifact produced by more gas going into physical solution in the tissues. An attempt to find morphologic evidence for oxygen poisoning in an active metabolic unit, the mitochondrial particles of alveolar cells of the rat lung, was not successful (263). Direct measurement of oxygen tension in the cerebral cortex of cats indicated an increased value during exposure to increased oxygen in the inspired air, but a threefold increase in the posthyperoxic period was explained by reactive hyperemia (141).

A puzzling consequence of breathing high concentrations of  $\text{CO}_2$  is the development of a bicarbonate deficit, a response just opposite to that which would assist in restoring the pH to normal value. Brown (42) restudied this problem in dogs and points out that the bicarbonate deficit is due to a rise in serum phosphate concentration. The mechanism, however, remains obscure. Tissue  $\text{CO}_2$  concentrations in rats exposed to high  $\text{CO}_2$  concentrations rose at a rate about  $0.036 \text{ min.}^{-1}$  (260) without appreciable difference between the three tissues studied, skeletal muscle, heart muscle, and brain. The barium-soluble  $\text{CO}_2$  in tissues, which has been of great importance in the calculation of tissue pH values showed a large variation in this study and raised once again the question of significance of the bound  $\text{CO}_2$  *in vivo*. Apneic oxygenation, earlier called "diffusion respiration", has been studied in man (114). The effects during progressive apnea are about the same as those previously reported in experimental animals except that the rate of rise of  $\text{CO}_2$  concentration was half that seen in the dog, and the human subjects were able to maintain oxygen saturation for as long as 30 to 45 minutes. Very high concentrations of  $\text{CO}_2$  can be tolerated without respiratory or circulatory depression if the effects of anesthesia and convulsions can be eliminated (117).

#### GAS EXCHANGE

One of the most original contributions to the study of respiratory gas exchange is the suggestion that oxygen transport through hemoglobin solutions represents a kind of facilitated diffusion. Wittenberg (277) used this hypothesis in his analysis of the mechanism of oxygen accumulation in the swim bladder of fishes. In an *in vitro* system he measured the diffusion of oxygen

through agar gels containing hemoglobin. The diffusion coefficient for oxygen was found to be 1.6 times greater through an oxyhemoglobin agar gel than through a similar gel in which the hemoglobin had been "inactivated" by carbon monoxide. The differences in this case are not great, particularly when the limits of accuracy of this type of measurement are considered. Scholander (242), on the other hand, has presented a much more complete picture and finds, under conditions of low pressure, that oxygen transport in the presence of hemoglobin molecules is eight times increased over that of straight diffusion. His technique measured steady-state gas flux across a millipore membrane that held hemoglobin solution in the pores. In this system the processes involved in the transport are two, a purely physical diffusion through the solvent, and a physicochemical event dependent upon hemoglobin and involving the kinetics and oxygen-binding properties of the hemoglobin molecule. The flux will depend on both the true coefficient of diffusion and on the solubility, in the case of oxygen, much enhanced by the presence of hemoglobin. In a steady-state situation the actual drop in oxygen concentration through the solution will depend on the shape of the oxygen dissociation curve, but the net effect is a handing down of oxygen molecules from one hemoglobin molecule to the next in a chain likened by Scholander to a bucket brigade. Exactly what role this process may play in normal gas exchange is not apparent, but it is interesting to observe that a recent analysis of red cell membranes reveals the presence of hemoglobin (8). The importance of myoglobin in facilitating oxygen transport from the blood capillaries to the contractile mechanism of muscle by this mechanism is clear, and it was found that this pigment, like hemoglobin, does indeed enhance oxygen transport *in vitro* (242).

The pulmonary capillary bed, by contrast with the rest of the pulmonary circulatory system, is pretty much the special province of the respiratory physiologist. In a careful study, Cander & Forster (56) sought to circumvent the criticisms inherent in the old inert gas methods of measuring pulmonary capillary blood flow. The insoluble gas helium was used to determine the dilution factor for the soluble gases, in this study  $\text{SF}_6$ ,  $\text{N}_2\text{O}$ ,  $\text{C}_2\text{H}_2$ , diethyl ether, and acetone. Sulfur hexafluoride was so insoluble that it behaved like helium, but ether and acetone were so soluble in lung tissues that exchange could not be measured at the alveolar level. Nitrous oxide and  $\text{C}_2\text{H}_2$  showed an initial rapid drop in alveolar concentration during breath-holding that was attributable to the solubility in lung tissues, and a subsequent slower fall in concentration that was a function of pulmonary capillary blood flow. With a factor for lung tissue solubility (57), and with the assumption that equilibration with lung parenchymal tissue is instantaneous, an expression was derived for expired alveolar gas concentration at any time after breath-holding. Experimental results were compatible with the theoretical derivations and provided numerical values for pulmonary capillary blood flow and lung tissue volume of 3.31 l./min./m.<sup>2</sup> and 606 ml., respectively. The uptake of cyclopropane (245) in man was studied, using Kety's equations as a mathematical



model. Although the experimental and theoretical predictions were in fair agreement, the body seemed to equilibrate somewhat more rapidly than predicted, explained by the authors as an underestimation by the mathematical model of the rate of delivery of inert gas to well-perfused areas of the body. The simultaneous elimination of acetylene and argon has been used to measure impaired diffusion (68) since the transport of the two gases is equal.

Thermal homeostasis between alveolar air and capillary blood is a necessary assumption to many calculations of respiratory gas exchange, and although the soundness of this assumption was earlier verified in experimental animals, during the past year man, too, was found to maintain a constant alveolar temperature even under extreme thermal conditions (233).

A detailed chemical study of the enzyme carbonic anhydrase (82, 83) reveals several findings of great interest. In examining the influence of ionic strength, it was found that the sole effect was in the conversion of the enzyme-substrate complex to the enzyme and final product. This suggests that the direct product of the enzyme catalyzed reaction is most probably bicarbonate, in contrast to the uncatalyzed reaction where carbonic acid is the chief product at pH 7. The function of the protein in carbonic anhydrase, in addition to providing specificity to the reaction, may be in the formation of a stable chelate of zinc enabling hydroxylation of zinc, and subsequently of carbon dioxide, to take place at lower pH than in the uncatalyzed reaction. Further studies on the purification of carbonic anhydrase place its molecular weight at about 31,000 and indicate that it is homogeneous (172) rather than being in two forms.

Inhibition of carbonic anhydrase with acetazolamide interferes sufficiently with the normal processes involved in  $\text{CO}_2$  exchange so that equilibration of alveolar air and pulmonary capillary blood is not complete at the end of the capillary (64), and until a new  $\text{CO}_2$  gradient from tissues to alveolar air is established,  $\text{CO}_2$  output falls (53, 239).

The exchange of  $\text{CO}_2$  between the circulating blood and the tissues of the dog's hindlimb (72) was studied, using  $^{14}\text{CO}_2$ , and subsequently applied to a discussion of the various local pools of  $\text{CO}_2$  that exist in the body (73). Studies of this type should assist greatly in closing the gap between mathematical models for turnover rate which treat the whole body as a single ideal system and the physiological complexity that it is. The  $\text{CO}_2$  stores of the body during voluntary hyperventilation decrease at a rate of about 161 ml. per mm. Hg fall in partial pressure of  $\text{CO}_2$  in the venous blood (264). The elimination of  $\text{CO}_2$  does not proceed at a single exponential rate, an observation entirely consistent with the concept of many types of storage pools exchanging at different rates. On the other hand, rate of elimination from the body also depends upon the distribution of ventilation to perfused alveoli, as can be illustrated by  $\text{CO}_2$  retention after occlusion of a pulmonary artery (259).

Short-term breath-holding with various inspired  $\text{CO}_2$  concentrations results in a final  $\text{CO}_2$  tension that relates linearly to the change in alveolar  $\text{CO}_2$

tension with time (152). This is consistent with the theory that the rate of exchange of  $\text{CO}_2$  is proportional to the  $\text{CO}_2$  gradient between blood and alveoli. Longer-term breath-holding requires precise knowledge of the change in lung volume (190). Output of  $\text{CO}_2$  into the lungs falls in the face of a sustained oxygen consumption, with a resultant shrinkage of lung volume and rise in  $\text{CO}_2$  tension because of the concentration effect. There is a progressive fall in  $\text{CO}_2$  output into the lungs caused by physicochemical factors that bring about a differential effect on the arterial and venous blood. Arterial  $\text{CO}_2$  tension is rapidly elevated because of a failure in clearance of venous capillary blood in the alveoli, together with the Haldane effect. Buffering capacity of the tissues tends to suppress the rise in venous  $\text{CO}_2$ ; and the Haldane effect, as oxygen is lost to the tissues, serves as a further buffer mechanism. It was predicted that at an exchange ratio of 0.33 the  $\text{CO}_2$  tensions of arterial and venous blood would be equal.

Tissue  $\text{CO}_2$  tensions estimated by subcutaneous gas pockets (108) in man provided a mean tissue tension of 41 mm. Hg, a value on the whole lower than ordinarily reported with this technique. In fact, some of the measurements in this study indicated values below arterial tension, probably because of a combination of temperature effects and reactive hyperemia. Carbon dioxide tensions in lymph (58) were found to be, for the mean, 18 mm. Hg higher than in simultaneous arterial samples and suggest that many estimates of tissue  $\text{CO}_2$  tension are too low.

*Diffusion.*—The pulmonary diffusing capacity at rest decreases significantly with age, and although it increases during muscular exercise in all age groups for equivalent degrees of oxygen consumption, it never achieves as high a value in older subjects as in younger subjects (91). The combined effects of age and exercise provide important data for the interpretation of mechanisms. It has been presumed that the decrease in diffusing capacity with age represented a decrease in pulmonary capillarity, but the increase during exercise indicates that the bed can be expanded. On the other hand, cardiac output decreases with age, and these authors argue convincingly for the importance of this fact in explaining changes in diffusing capacity with aging, both at rest and during exercise. Increase in lung volume will increase diffusing capacity (191), but this factor was not changed during exercise (91). By contrast, change of cardiac output alone was found not to alter the value of the diffusing capacity (229), but the latter was discovered to be very sensitive to change of ventilation. In fact, steady-state diffusing capacity increased by voluntarily increasing ventilation to the same degree as that achieved during exercise, but breath-holding diffusing capacity was not altered by hyperventilation, although it is increased during exercise. Burrows *et al.* (49, 50) have found evidence of non-uniform pulmonary diffusion, best expressed as a variable ratio of diffusing capacity to lung volume. In the normal lung the net effect is that 6 to 18 per cent of the lung volume behaves as if it contained 12 to 46 per cent of the diffusing capacity. In disease and possibly in exercise, this concept may explain the different

results obtained by different methods for measuring diffusing capacity. Epinephrine causes a slightly increased pulmonary diffusing capacity for oxygen, but 5-hydroxytryptamine, which also has pulmonary vasomotor action, is without effect (93). Both exercise and hyperpnea may increase the blood-gas interface area (177, 251), but the differences in results between the various methods remain unsolved.

A general solution of diffusion problems through an electrical analogue process was sought by Niesel & Thews (197), and a rebreathing method for the measurement of carbon monoxide diffusing capacity was described (168).

*Ventilation: perfusion relationships.*—Remote from a direct experimental approach, the relationships of ventilation and blood flow in the lung tax the deductive ingenuity of the physiologist. In a cleverly designed experiment, Haab, Piiper & Rahn (124) examined the distribution of ventilation:perfusion ratios in the lung as a component of the alveolar-arterial oxygen tension difference. The contribution of the distribution component had been shown by Farhi & Rahn to vary with the fraction of nitrogen in the inspired air. To keep the alveolar oxygen tension constant, since both the diffusion and shunt components depend on this value, the alveolar-arterial oxygen tension difference was measured breathing atmospheric air at sea level, and pure oxygen at a reduced ambient pressure adjusted to provide an inspired oxygen tension equivalent to that of air breathing at sea level. No difference in the alveolar-arterial oxygen tension difference was found between these two experiments, suggesting that the distribution effect does not contribute importantly under normal conditions. The original theoretical assumptions about the type of statistical distribution of ventilation:perfusion ratios among the alveoli of the lung may have been erroneous.

Deliberate alterations of either ventilation or blood flow by constricting the bronchi or pulmonary artery result in important consequences of gas exchange (268, 269, 272). By measuring simultaneously the argon, carbon dioxide, and oxygen concentrations following a single breath of argon in air, bronchial constriction resulted in a low expired argon concentration, a fall in the respiratory exchange ratio, and a distinctive change in the shape of the pattern of gas concentrations against time. Specifically, the slope of the inspiratory phase increased without change in end-tidal value, and a pip, or notch, appeared in early expiration. After arterial constriction there was no change in end-tidal argon, the exchange ratio increased, and the single-breath excretion curve was flattened during expiration and often showed a pip at the beginning of inspiration. With combined obstruction of bronchi and blood vessels, the respiratory exchange ratio fell, an expiratory pip appeared, and the expired argon concentration was low. Alterations of the ventilation:perfusion ratio were used to explain all these findings, with the increased slope of the expiratory curve representing a slow washout of dead space with a consequently slower approach to the alveolar value. Certainly the alveolar dead space increases with vascular occlusion (145), and West & Hugh-Jones (269) emphasized the change in mechanical characteristics of

the obstructed lobe that change the sequence of emptying from various regions resulting in the pips and other deviations from the normal contour of single-breath concentration curves.

With prolonged exposure to positive radial acceleration, hypoxemia developed even when 100 per cent oxygen was breathed (20). It was postulated that this could best be explained by a large intrapulmonary shunt, but the whole effect could be abolished by vagotomy. Artificial respiration also resulted in an increase in the alveolar-arterial oxygen tension difference (113), presumably also due to a redistribution of ventilation:perfusion ratio. Deviations from the normal distribution pattern assume great importance in certain diseases, most notably pulmonary emphysema (221). Interstitial disease of the lungs results in a distribution anomaly, but the ratio in this case is exclusively dependent on regional alterations of ventilation, for the blood flow remains uniform (220). Epinephrine and aminophylline, although they are potent bronchodilators, cause a drop in arterial oxygen saturation in patients with pulmonary disease, indicating an overriding importance of the vascular changes with resultant increase of venous admixture (127).

In a region of the lung where the ventilation:perfusion ratio is reduced to zero, for example by completely occluding the bronchus (268), the oxygen tension in that region falls at a rate that correlates surprisingly well with blood flow. The technique of extracorporeal blood oxygenation and circulation provides an interesting tool for the exploration of ventilation:perfusion ratio effects (175).

*Intrapulmonary mixing and clearance.*—Since the finding that a single exponential term was not a satisfactory expression for the time course of clearance of nitrogen from the lungs, much work has been done to derive a mathematical model consistent with the physiological observations. An attempt was made to quantitate the effect of a single dead space on lung clearance rate in a model containing two parallel ventilating compartments (267), and also in models with either common or separate dead spaces (275). The regional deposition of aerosol in the respiratory tract, with consideration of respiratory pauses and mechanical mixing of gas flow, was treated mathematically by Altschuler (5).

Intrapulmonary gas mixing as inferred from nitrogen washout has been confirmed to be less efficient in the older age group (67, 240). Using the eliminations of both helium and nitrogen, uniform values for both slow and rapid uneven ventilation were found in all normal subjects (261), but smaller values for unevenness were found for helium than for nitrogen. When the theory of "periodic" ventilation was used, far greater values for the degree of unevenness were found.

The contribution of tissue nitrogen elimination to the analysis of pulmonary nitrogen clearance curves was studied by Bouhuys (34) and found to be less important than the inherent errors of the graphical analysis. Others have refined the analysis of intrapulmonary mixing by a back-control of the measurement that assesses contribution of tissue gas to the washout curves (194).

Postural changes influence the clearance rates of nitrogen from the lungs and are important from both a theoretical and practical standpoint. Nitrogen clearance is known to be retarded in the lateral decubitus position, but not in the supine. In a study of the nitrogen clearance rates of right and left lungs in different positions, Lillington *et al.* (170) found that the clearance rates of both rapidly and slowly ventilated components of a lung were only half as great when it was in the superior position as they were in the same lung when it was supine. Low clearance rate in the superior lung was the responsible factor for the change in total lung clearance.

Agents that influence bronchomotor tone may be expected to alter the pattern or distribution of ventilation. In normal subjects epinephrine was found to be without effect on nitrogen clearance, but hexamethonium increased the value of the clearance index in three of four normal human beings (35). Histamine given intravenously did not affect nitrogen clearance, but when inhaled it had a retarding effect (32).

#### MUSCULAR EXERCISE

In a careful evaluation of all the chemical factors involved in both the systemic and cerebral circulations, Lambertsen *et al.* (160) formulated important conclusions about respiratory control during exercise. If, instead of breathing air during exercise, oxygen at 2.0 atm. pressure was breathed, the arterial  $P_{CO_2}$  and pH, both normally lowered, were restored to normal, and the ventilation was decreased. Cerebral venous pH remained low, even though there had been a readjustment in the systemic circulation towards more normal acid-base balance. There was a negative correlation of ventilatory response with the  $P_{CO_2}$  in both the arterial and cerebral venous blood, but there was a highly significant, positive correlation with the hydrogen-ion concentration in both arterial and cerebral venous bloods. Perret (210), in a somewhat similar study, reached the same conclusion regarding the importance of acidemia to respiratory drive in exercise but suggests that there is a hypoxemic chemoreceptor drive relieved by oxygen administration, an explanation Lambertsen found unacceptable because there was normally no fall in arterial oxygen tension. Friehoff (110) found a consistent fall of arterial oxygen tension during exercise in his group of normal subjects, but the change was of the order 5 mm. Hg only, and the most important effect was an increase in the alveolar-arterial oxygen tension difference, this value very nearly tripling. The "fixed" acid causing the pH change (160) may well be lactic (210), and its rate of production is probably reduced by oxygen at high tension, a satisfactory explanation for the modification of ventilation. The rise in blood lactic acid is more closely related to the intensity of exercise than to its duration (87). Even within a period of one minute there is sufficient lactic acidemia to result in a blowing off of  $CO_2$  during the immediate recovery period in the amount of 2 to 3 per cent of total body stores (6). Intermittent muscular work results in low lactic acid levels and increased mechanical efficiency (65), explained in part by the important function of myohemoglobin as an oxygen store system (10, 11). In the older

age groups the recovery rate is poor compared to that seen in younger subjects (7).

A study of the cardiorespiratory response to exercise in patients with anemia demonstrates how well the adaptation to this deficiency is met (253). Through compensatory mechanisms, the anemic individual at rest handles blood-gas transport and exchange adequately. During strenuous exercise the hemodynamic adjustments become inadequate, and a fall in oxygen intake and carbon dioxide production results. Despite this the arteriovenous pH difference is not exaggerated when compared with normals, suggesting that there may be a lag in the release of tissue acid metabolites.

It is generally agreed that proprioceptive stimuli are important components of the ventilatory drive during muscular exercise, but if alveolar gas tensions at the involuntary breaking point of breath-holding are indices of chemoreceptor drive, then it is surprising that the breaking point during exercise is characterized by higher  $\text{CO}_2$  tensions and lower  $\text{O}_2$  tensions than at rest (12). This increased tolerance is still apparent immediately after work. Nonparalyzing doses of curare given to normal individuals caused an increased ventilatory response to muscular exercise (205), which was attributed to an effect of motor innervation on the respiratory center.

Respiratory work increases during muscular exercise, although the relationship between intraesophageal pressure and exercise was more linear than that between respiratory work and exercise (202).

The achievement of maximum oxygen uptake during exercise is not determined by a limit for the diffusing capacity but is more likely to be of circulatory origin (91). Oxygen uptake reaches its asymptote more slowly than does the heart rate (280). During exercise there is a redistribution of the blood volume towards the central reservoirs. Braunwald & Kelly (38) found that for a mean rise of oxygen consumption from 144 to 1011 ml./m.<sup>2</sup> the cardiac index rose from 3.42 to 7.99 l/min./m.<sup>2</sup> and central blood volume increased by 141 to 745 ml. in eight subjects. This increase in the central blood volume, possibly related to the change of peripheral vascular tonus (185), is doubtless of importance in expanding the pulmonary capillary bed to increase the diffusing area.

#### COMPARATIVE PHYSIOLOGY

The enzyme carbonic anhydrase is distributed throughout the mammals in such a way that the smallest animals have the highest red cell concentration of the enzyme (163). Though the enzyme is present in excess, even in those animals with the highest metabolic rates, the direct correlation of enzyme concentration with metabolic activity is of interest. It is quite possible that the rapid hydration of  $\text{CO}_2$  with its influence on the dissociation of oxyhemoglobin (Bohr effect) is of importance in oxygen delivery to the tissues. The pH sensitivity of hemoglobins from a variety of mammals also correlates inversely with body size and, therefore, directly with metabolic

activity (224). In the alligator red cell, carbonic anhydrase is about one-twentieth that found in the dog (276).

Insects have no true ventilatory system but have a highly developed tracheolar system that functions through both a diffusion mechanism and the volume change consequent to the respiratory exchange ratio. Each spiracular opening along the body wall contains two groups of muscle fibers that keep the aperture closed much of the time. Hoyle (137) showed that there are two motor axons to the system, one of which is fast and produces a twitch, the other slower, and also producing a twitch, but of only about one-third the magnitude of the fast-fiber response. Periodically, there is a relaxation of the muscle units, and the elastic forces of the hinge mechanism allow the spiracle to open. The stimulus for the relaxation of the muscle is nervous in origin and comes from an abdominal site. There is no evidence for a peripheral sensing mechanism, but in low concentrations,  $\text{CO}_2$  will relax a closed muscle, even in the face of continued nerve stimulation (138). Carbon dioxide gas appears to act directly in this case on events of the neuromuscular transmission sequence, with a resultant decrease in magnitude of the junctional and twitch potentials.

Gill ventilation in the dogfish and skate is accomplished by the development of both negative and positive pressures in the mouth (140). Water entering on one side of the mouth exits by gills on the same side and except for brief periods there is an almost continuous flow across the gill area. In the marine teleosts there is a buccal pressure pump and spiracular suction pump (139). The swim bladder, of great importance to buoyancy in fish, also has a respiratory function. In *Notopterus* (Lacepede), surfacing at intervals is necessary for respiration, but if this is prevented the air reservoirs of the swim bladder are called upon (86). Snails can alter their oxygen consumption in accordance with the available oxygen in the water, but there is great variation amongst species and some do not decrease their demands until a critical tension is achieved, which may be very low (24).

Avian respiration is complicated by a vast array of air sacs that communicate with the lungs, bronchi, and bones of the body. Shepard *et al.* (250) have followed the course of air through this system in the anesthetized chicken and determined the direction of movement to be from mouth to lung to air sac to mouth, with complete valving.

#### HEMOGLOBIN

The very elegant studies on the structures of hemoglobin (211) and myoglobin (146) offer hope for a more complete understanding of gas transport, but unfortunately it is still necessary to conclude that "little can be said of the relation of structure to function." Mercapto-mercapto interactions of normal human hemoglobin seem to be closely associated with the heme groups and are dependent on the structure of the protein constituent because the reactions vanish with denaturation (192). Synthetic hemoglobins are useful for studying the heme-heme interactions and the



characteristics that determine oxygen affinity (230). If the hemoglobin molecule is split with urea, oxygen affinity decreases and the sigmoid shape of the dissociation curve changes (231). Increased concentration of salt decreases the oxygen affinity of a hemoglobin solution (47), an action possibly related to the Bohr effect and perhaps of great importance in some abnormal states.

The effect of environmental pH is important to oxygen transport, in part because it influences the affinity of hemoglobin for oxygen. The primary oxygenation-linked acid groups were identified as sulfhydryl groups of cysteine residues (224) rather than imidazole or ammonium groups as previously thought. A remarkable correlation was found among the terrestrial mammals, such that the smaller the animal, the larger was the number of cysteine residues per hemoglobin molecule and the larger was the number of protons discharged during oxygenation. This observation provides a satisfactory explanation for the inverse correlation between body weight and oxygen affinity that is known to exist. Hemoglobin was found in the red cell membrane (8) although its function there is uncertain. The abnormal hemoglobins of the common hemoglobinopathies were studied by constructing *in vivo* oxygen dissociation curves (227). The dissociation curve was normal in patients with A—C and A—S hemoglobin and in one instance of a patient with S—A hemoglobin and thalassemia. In five patients with no normal hemoglobin, there was arterial oxygen unsaturation adequately explained by the shift in the dissociation curve to the right, but this was thought to be caused by a reduction of pH in the red cell interior.

#### CHEMISTRY OF LUNG

Brief mention is made of several contributions to present day understanding of lung function that are without direct reference to respiration per se. The lung is not only a fat depot (149) but is important in the synthesis of fat and fatty acids (182, 281). These poorly understood functions may be of considerable importance to a complete study of gas exchange. Histamine is both liberated from pulmonary tissue and removed by it (66). Both the elastin and collagen content of the lung increase with age, but lipid content decreases (39, 40). Lung tissue adjacent to bullae contains a low concentration of elastin.

#### RESPIRATION IN DISEASE

Although it is quite impossible, as well as inappropriate, to review all the contributions to the field of clinical respiratory physiology, several references are briefly noted below.

The respiratory insufficiency of chronic pulmonary emphysema can be alleviated by mechanical respirators although they do not all work equally well (142). A new carbonic anhydrase inhibitor (dichlorophenamide) serves to increase alveolar ventilation and improve blood gases in these patients, apparently by an action on the respiratory center (193). Half of the patients

with pulmonary emphysema studied in one series were found to have an elevated blood ammonia level (94), possibly due to a damaging effect of long-standing hypoxia on liver function. In this group there was an apparent correlation between alveolar ammonia and minute volume of ventilation. In cirrhosis of the liver there is a high blood ammonia level as a result of primary liver disease, and this group is characterized by chronic hyperventilation (129). Another cause of hyperventilation in such patients may be the arterial unsaturation which is commonly present, possibly because of a venous admixture (273).

In ankylosing spondylitis, there is a relative overexpansion of the lower and underexpansion of the upper portions of the lung, producing a hypoxemia and hyperventilation primarily caused by a ventilation:perfusion ratio abnormality in the lungs (222). Kyphoscoliosis, on the other hand, is characterized by chronic hypoventilation as the body strives to minimize the work of breathing with a frightfully deranged apparatus (25). In pulmonary fibrosis, the work of breathing is increased because the lungs have a decreased compliance secondary to change in interstitial tissue (270). In the Hamman-Rich syndrome, there is an increased dead space and ratio of dead space to tidal volume probably secondary to focal emphysema (135). Pulmonary embolism serves to alter the blood distribution in the lung sufficiently to increase the physiologic dead space (226).

In unconscious patients, the tongue is pushed against the pharyngeal wall when the neck is flexed and the mandible drops back (236). The control of ventilation with a volume-limited, pressure-variable respirator<sup>1</sup> is described (98). Greene (121) cautions on the too prompt relief of respiratory acidosis with tracheotomy. The interesting syndrome of obesity and hypoventilation continues to merit reports (125), the current conclusion being that the increased work of breathing is causative.

An international study comparing maximum breathing capacity values between Danish and English subjects indicated higher normal values in Denmark (206). There were fewer smokers in Denmark and more symptoms of respiratory disease in England. Airway resistance in bronchial asthma is increased even when there are no symptoms (235). The onset of dyspnea in normal subjects and patients with cardiorespiratory disease is determined in some measure by the respiratory pressures achieved (200, 201). Hyaline membrane disease in infants reduces pulmonary compliance, possibly because of reduction in the number of participating units (122).

In myxedema, there is no evidence of disease of lungs or thorax, but hypoventilation is commonly present (274). The authors believe there may be malfunction of the muscles of respiration or of neuromuscular co-ordination.

#### METHODS AND PROCEDURES

Many new or modified methods of interest to the respiratory physiologist appeared during the past year. Relatively new is the increased appreciation

of the technique of gas chromatography (92, 143). New and important modifications of the oxygen electrode (61, 228) appeared, including a technique for recording the oxygen tensions continuously with a catheter electrode (155). Several variants of older procedures for measurement of  $\text{CO}_2$  in respiratory gas (54), and in blood and tissue (55, 99, 132, 203), were reported including the description of a tonometric apparatus for constant flow gas equilibration (126). Alveolar ammonia is of growing interest, and a detailed method of analysis was outlined recently (216). A breath-by-breath sampling system for alveolar gas that allows alteration of the duration of the sampling period was described by Lambertsen & Benjamin (161). Methods for the study of respiration in small laboratory animals (37, 95), aquatic animals (28), and isolated guinea pig lungs (81), and a new perfusion method (90) that promises sustained performance in the isolated lung were presented. There are new methods for measuring intraesophageal pressure (134, 241) and the compliance and non-elastic resistance of the chest (195, 248), and some modifications of old methods for measuring the lung volumes (88, 184, 279). Energy expenditure may be estimated from pulmonary ventilation (107), a technique of interest in field work. An analogue computer was used to measure respiratory depression (23), and an alveolar  $\text{CO}_2$  tension control system was used to magnify respiratory depression by drugs (162). The control of tidal volume is of great importance in many experimental designs, and a simple method of control has been described by Lichtneckert (169). The diaphragmatic electromyogram may be used to monitor ventilation (105).

Of the great variety of pulmonary function tests available, certain basic measurements are gradually emerging as the most useful. The relationship of expiratory flow velocity to the maximum breathing capacity (36, 178, 188, 278) is well established, but the importance of instrumental resistance needs occasionally to be restated (284). Normal values for the common measurements of pulmonary function may vary  $\pm 20$  per cent for normal, young male subjects (136), and new values for normal children were presented (254). Throughout the day there may occur significant variations in forced expiratory volume and forced expiratory vital capacity (167).

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## DIGESTIVE SYSTEM<sup>1,2</sup>

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The customary disclaimer that this annual review takes a limited view of the field is issued and may be promptly discounted. Limitation of space aside, the availability of numerous abstracting and translating services which have breached linguistic curtains has only increased the embarrassment of riches, and progress does not necessarily proceed by calendar years. The biases of interest are more relevant, and the reader has the right to know that this reviewer approaches the search for order in gastrointestinal function in the hope that it will shed some light on the disorder of disease—a hope usually not satisfied.

Several topics have been completely omitted, salivary secretion for one since it has been reviewed in considerable detail in the last two *Annual Reviews*. Gastrointestinal physiologists in general have not appreciated the accumulation of information now available concerning central control of digestive function; their attention is directed to Eliasson's succinct review in the *Handbook of Physiology* (1). Active transport throughout the gut is stressed elsewhere in this issue. Hunt's review of gastric secretion and gastric emptying in man (2) illustrates the value of relating several of this organ's manifold functions. Concentration on the themes of food intake, pyloric gland area, and the movement of plasma proteins into the gut reflect actively growing areas of interest as well as reviewer bias.

In many areas of physiology, "molecular-biology" is the current catch phrase. In the gastrointestinal tract we are still concerned with organ physiology, and it appears to me that we are likely to continue this profitably in the immediate future.

### REGULATION OF FOOD INTAKE

In recent years it has become a commonplace among interested investigators that the study of food intake at the physiologic level is part of an inquiry into the homeostasis of energy regulation, and thus an established, indeed almost respectable, area of experimental investigation. This subject has been touched upon in this section of the *Annual Review* in more than a cursory fashion, however, only twice in the last ten years (Grossman, 1950; Mellinkoff, 1957).

The broad outlines of the central nervous system control of the complex act of eating have been defined by Brobeck (3) in terms of the facilitation and

<sup>1</sup> This review is based upon a survey of material concluded in May 1960.

<sup>2</sup> Among the abbreviations used in this review are: CAH (carbonic anhydrase); GHC (gastric hunger contractions); IF (intrinsic factor); and PGA (pyloric gland area).

inhibition of "feeding" reflexes by anatomically discrete hypothalamic centers (ventromedial nuclei = "satiety center"; lateral nuclei = "feeding center") integrating their activity into classical Sherringtonian neurophysiology.

Along with this approach has grown the feeling, almost hardening into dogma, that the outstanding still unsolved problem is how the hypothalamic regulatory centers receive their appropriate cues, that this is a problem in information theory.

Among the proposed but in this reviewer's opinion (4) still to be firmly established signals to the brain have been: (a) availability of blood glucose, (b) concentration in the blood of a circulating metabolite derived from or related to fat stores, (c) pattern of available amino acids in the blood, (d) water concentration in body tissues, and (e) specific dynamic action of ingested food. Less controversial because less studied have been the oropharyngeal metering of ingested volume, and inhibitory impulses from the distended stomach mediated via the vagus. For the sake of harmony almost all workers accept a multifactorial approach.

Hervey (5), in simple but theoretically elegant studies, has furnished some evidence for the first time that a "feedback" mechanism may indeed be operant in feeding behavior, that the peripheral stores relay information regarding their inventory to the hypothalamic regulatory mechanism. Lesions were made in the ventromedial nuclei of the hypothalamus of one of pairs of parabiotic rats with resultant hyperphagia and obesity of the operated member of the parabiotic union; the non-operated member responded by eating less and becoming thin. Lesions subsequently made in the hypothalamus of the partner resulted in the expected hyperphagia. The implication of these experiments is that the hypothalamus of the normal animal responds to the operated animal's overfeeding. Hervey favors some maintained change within the animal, rather than fluctuation in blood levels and inclines towards Kennedy's "lipostatic" concept. A striking finding in these experiments was the observation that the excess food eaten was laid down as fat only within the carcass of the operated animal, a bit of evidence against the concept that impulses from fibers in adipose tissue inhibited the normal partner. Cutaneous adipose tissue of hereditarily obese mice behaves normally when transplanted into normal mice (6).

A number of other studies during the past year have dealt with the central aspects of feeding. Morgane & Kosman (7) report that lesions of the rhinencephalon, primarily pyriform lobe and amygdaloid complex, in the cat lead to hyperphagia and weight gain. Neodecortication had no effect on this procedure. Since in most schemes the ventromedial nuclei ("satiety center") are believed to inhibit the lateral nuclei ("feeding center"), it is of interest that Brooks (8) in a study of simultaneous records obtained from both areas in unanesthetized animals failed to find evidence of interaction. Further evidence that the lateral nuclei could be depressed directly, rather than via the medial, is presented by Epstein (9) in a demonstration that several sympathomimetic amines capable of suppressing normal appetite also suppressed

the hypothalamic hyperphagia of rats. Yet Brobeck, Larsson & Reyes (10) have reported that amphetamine gives rise to increased electrical activity in the ventromedial hypothalamic nuclei, either directly or indirectly from areas of the cortex.

One of the difficulties attendant on Mayer's concept that "gluco-receptors" in the hypothalamus regulate food intake by way of information received from the available carbohydrate stores of the periphery has been the differences in carbohydrate metabolism between the brain and the periphery. Rowe and his associates (11) have reinvestigated cerebral blood flow and carbohydrate utilization in man before and after eating. While in peripheral tissues there was a decreased rate of glucose utilization in the fasting state (decreased  $A-V\Delta$ ), the brain on the other hand extracted roughly the same amount of glucose in the fasting state as at the peak of arterial glucose concentration following the test meal, although it may be true that variations in a small center may not be demonstrable in a study of the total brain metabolism [Forssberg & Larsson (12)].

The tenor of recent discussion has been to minimize the role of gastric hunger contractions as cues for food intake, not only because it is difficult to rationalize their role in long-term regulation of any precision. It is of interest, therefore, that Mayer and his colleague Sudsaneh have attempted in a series of papers (13, 14) to relate gastric hunger contractions (GHC) to concomitant metabolic events and to hypothalamic control using the rat with water-filled balloons in the stomach. Small amounts of intravenously administered glucagon, epinephrine, and norepinephrine inhibited these hunger contractions, as did exposure to heat or cold. These changes bore no relationship to blood sugar levels, and norepinephrine is known not to affect carbohydrate levels. The glucagon inhibition was preceded by a fall in plasma phosphate, however. Gastric hunger contractions occurred in hypothalamic aphagic and hyperphagic animals quite similar to those occurring in the normal animal. Heat, cold, and epinephrine inhibited in both types of animals. More cold was required in the hyperphagic animals, and glucagon produced much less inhibition in hyperphagic rats whether they were obese or reduced in weight. These workers believe that the ventromedial "glucoreceptive" hypothalamic area not only modulates the lateral area, but affects food intake via gastric hunger contractions by way of connections through the Bundle of Schutz with the dorsal nucleus of the vagus. However, these contractions have considerable peripheral autonomy occurring in the transplanted denervated pouch, and their importance in regulation of intake to energy needs over any significant period of time has yet to be established.

It has been known for a long time that there is a close correlation between food intake and water intake in normal animals and in animals with hypothalamic lesions, but systematic study of these interrelations, especially over long periods of time, is badly needed. Cizik has remedied this in part by a report of a protracted period (7 years) of observation on water and food intake in the dog (15). Water intake was adjusted in this period to the water and salt content of the diet. Food deprivation led to a prompt drop in water

intake, but not its abolishment. Continued food deprivation led to a gradual increase in water intake, related by him to urinary salt losses. This area of the relationship of osmolality of body fluids to food intake appears a profitable one for future research.

### GASTRIC SECRETION

*Fine structure of the parietal cell.*—Lingering doubts about the cellular locus of hydrochloric acid formation have not inhibited continued electron microscopic study of the parietal cell. In the mouse, Hally (16) has investigated the branching system of intracellular canaliculi, which is lined by numerous microvilli. An interesting feature was the cytoplasmic vacuoles found mainly in relation to the intracellular canaliculi, which vacuoles do not form a reticulum. Vial & Orrego (17) have seen these vacuoles in a number of species and noted unfolding changes in them with histamine stimulation. To an amateur the changes suggest "reverse" pinocytosis: a suggestion which must be greeted with horror by those who consider that pinocytosis cannot provide a satisfactory explanation for active transport.

Despite what Davenport has called the "unhappy history" of carbonic anhydrase (CAH), this enzyme continues to fascinate and preoccupy many investigators. The older method for staining this enzyme (Kurata's) has been subjected to much criticism. It is significant that Hausler (18) reported a new method early last year, which is inhibited *in vitro* by acetazolamide, and with which Schiebeler & Vollrath (19) have demonstrated CAH in the gastric mucosa of man, rat, guinea pig, and cat in the form of an intracellular network in close proximity to intracellular canaliculi. Vollrath (20) also has noted the time of the appearance of stainable enzyme in embryonic rat, guinea pig, and cat. Whatever the limitation of the effect of the CAH inhibition by acetazolamide on the secretion of  $H^+$  by the isolated membrane, which this reviewer suspects is a limitation of the dose range explored *in vivo*, inhibition in dog and man has been now apparently followed by that of the rat (21). Miscellaneous reports indicate that sympathectomy (21) increased CAH activity of the rat, while insulin (22) increased CAH staining in the same animal. Vagotomy is said to be without effect.

*Potassium secretion.*—Continuing their studies of  $K^+$  in the isolated frog mucosa, Harris & Edelman (23) have presented evidence that a small but significant component of active potassium transport against the electrochemical-potential gradient occurs.

The actual concentration and a complete account of  $K^+$  in gastric juice have been controversial for a long time. In most studies the range of variation of  $K^+$  concentration has been small although the range of acid secretion varied greatly. The simplest explanation has been that the concentrations of  $K^+$  in the acid and nonacid components of the gastric juice are equal, and have been reported to be 7.4 m. eq. per l. by Gray & Bucher (24) and estimated at 10 by Hunt (2). Colcher & Hollander (25) have presented evidence of greater variability in  $K^+$  of vagotomized canine pouches stimulated with histamine, with values ranging from 1.6 to 11.2. In most instances the rises

above plasma levels occurred directly after initiation of stimulation. This would seem to resemble the initial transient stage of salivary secretion in which  $K^+$  is lost from the gland into the blood and saliva when a previously inactive gland is activated (26). When rates of gastric secretion were stabilized by repeated 10-minute injections of histamine, the range of potassium variation was only 2.8 m.eq. per l., but following a single large dose was 8.4 m.eq. per l. While the initial large rises in  $K^+$  concentration are clearly to be related to phenomena occurring at the onset of activation of the gastric glands, these experiments do not fully answer the question of the concentration of  $K^+$  being secreted in the steady state by one or more sources in the gastric mucosa. The results of the repetition of these experiments in the same laboratory following a single injection of histamine in the dog (27) and in man (28) were consistent with the experiments just cited. Riddell and his co-workers (29), on the other hand, have described a significant direct relationship between the rate of secretion of gastric juice and potassium concentration in man, resting or stimulated by histamine or insulin. The well-known inverse relationship between sodium and hydrogen was again demonstrated.

*Hydrogen-ion regulation.*—The great debate on the regulation of  $H^+$  concentration in gastric juice has been posed in the past on an either/or basis: either a dual secretion, or back diffusion of  $H^+$  across the gastric mucosa. In a study *in vivo* of salt and water movement across the resting stomach, Bornstein *et al.* (30) concluded that both substances move in the direction of their chemical potential gradients. In several experiments the amount of  $Na^+$  transported from blood to lumen equaled the amount of  $H^+$  lost from the lumen, certainly consistent with the back-diffusion hypothesis of Teorell. I would favor the point of view advanced recently by several reviewers (2, 31) that these opposing views are not necessarily mutually exclusive: not either/or, but rather "to what extent does secreted acid diffuse back across the secreting gastric mucosa under various normal circumstances?" would seem, as Hunt has suggested, the pertinent question.

Lambling and his colleagues (32), in a paper seen only in abstract, have been approaching the problem of the primary alkaline secretion of the stomach by studying the buffer capacity and electrolytes of patients with pernicious anemia in the light of the interesting concept that these stomachs retain the ability to secrete the alkaline component after atrophy of chief and parietal cells.

*Gastric secretion and surface epithelial cells.*—While the study of the fine structure of the parietal cell has continued with confidence that this is the acid-secreting cell, the controversial suggestion by Rehm that the surface epithelial cells are required for acid secretion has been disturbing, especially the notion that the  $H^+$  and  $Cl^-$  ions are secreted by different cells of the gastric epithelium. Davenport & Allen (33) have shown that the mouse stomach blotted so as to remove 97 per cent of surface epithelial cells can still secrete hydrochloric acid and be inhibited as does the normal.

*Gastric mucosal potentials.*—Last year's reviewer, Hogben, himself an accomplished investigator of the isolated mucosa and its electrical phenomena,



strikingly pointed up the dilemma between the failure of a constant association between the generation of a gastric potential and the secretion of  $H^+$  on the one hand and the necessity of formulating any consistent analysis in terms of general electrophysiology. Harris & Edelman (34), continuing their studies of the role of increasing the concentration of potassium on the nutrient side ( $K_n$ ) of the isolated mucosa and reducing  $pd$ , could uncouple the relation between e.m.f. and  $H^+$  in the presence of both histamine and thiocyanate. Extending their studies more recently (35) with further analysis of the electrical properties of the mucosa, they believe that elevating the  $K_n$  depresses active chloride transport while sustaining or enhancing the rate of hydrogen-ion production. Chandler *et al.* (36), having noted that the close arterial injection of potassium chloride also produced a decrease in potential difference and a decrease in electrical resistance in the dog, have been unable to ascribe this to anoxia although they noted blanching of the mucosa.

The problems of relating the gastric mucosal potential to the secretory processes are also pointed up by the study of Dennis *et al.* (37) who measured a significant potential difference across the pyloric gland area *in vivo* in the dog. Since current belief favors the concept that the fundic  $pd$  is related to chloride transport, it may be that a similar  $Cl^-$  pump exists in the pyloric gland area, but its relation to secretion in that area has yet to be demonstrated; while the chlorine concentration of the viscous fluid of this area is variable, it is usually well above plasma levels (up to 145 m.eq. per l.).

#### ROLE OF THE PYLORIC GLAND AREA

At the organ level, current interest in acid secretion, from physiologic and clinical points of view, centers around the function of the pyloric gland area (PGA). Grossman (38) has succinctly presented the reasons for preferring this term to the less clearly demarcated one of "antrum", and his terminology should find wide acceptance.

*Preparation of gastrin.*—Gregory & Tracy (39) have been engaged in preparing a potent gastrin from hog pyloric gland mucosa which is free of vasodepressor material. A striking property of this gastrin, which did not stimulate pepsin output, is that in contrast to older preparations, the subcutaneous route of administration was effective, whereas a single intravenous dose was ineffective in dogs with gastric fistulae and denervated gastric pouches. It is with this method of extraction that they together with French & Sircus (40) have been able to prepare a gastrin-like substance from a pancreatic tumor in a case of peptic ulcer with diarrhea and excessive gastric acid secretion (Zollinger-Ellison syndrome); they have been unable to extract this material from normal hog pancreas.

*Control of gastrin release from the pyloric gland area.*—Woodward (41) in summarizing his own and other recent contributions to the physiology of the PGA has pointed out the well-verified critical role of local pH of 1.5 or less in inhibiting the release of gastrin by chemical and mechanical stimuli of the PGA and, as will be emphasized below, this holds true for vagal stimulation

as well. This homeostatic mechanism within the stomach may thus control the cephalic and gastric phases of gastric acid secretion (for pepsin secretion also see below). It probably also influences that portion of the intestinal phase of acid secretion which may be mediated by the PGA release of gastrin stimulated by absorbed secretagogues. In this context it may not be amiss to point out that the effectiveness of local low pH's in the PGA in inhibiting the release of gastrin is dependent in part on vagal innervation, as well as on vagal stimulation.

*Vagus and gastrin.*—Clarification of the role of local acid in inhibiting the release of gastrin from the pyloric gland area has contributed to the clarification of another long-standing and important question: does the vagus release gastrin? Uvnäs suggested in 1942 (42) that during the cephalic phase of gastric secretion the vagus does in fact release gastrin from the pyloric region of the stomach. This has been energetically debated in the intervening years, the failure of sham feeding to stimulate a Heidenhain pouch being considered important evidence against this possibility. The intervening series of experiments which demonstrated that acid in the pyloric gland area leads to an inhibition of the release of gastrin necessitated experimental reinvestigation of the basic question posed above. This has now been done in a characteristically thorough fashion by Thein & Schofield (43), who prepared vagus innervated pouches of the pyloric gland area in dogs fitted with either Heidenhain pouches, or transplanted pouches of the gastric corpus. Sham feeding resulted in vigorous secretion within the Heidenhain pouch and transplanted corpus pouches; in the latter, marked potentiation took place with urecholine. Motility studies indicated that this was not related to antral motility, but clearly was caused by the release of gastrin from the pyloric gland area by the vagus in the absence of local acid. Under the condition of their experiments, the amount of gastrin released by sham feeding was enough to sustain secretion at rates that compared with the peak of the response to a regular meal by a similar pouch in dogs with the antrum in normal continuity.

In a series of simple and clear experiments, Gregory & Tracy (44) have extended this line of study by investigating the secretory response of denervated gastric pouches to feeding, intragastric stimulation, and the effects of concurrent administration of parasympathomimetic agents (urecholine and carbachol) in dogs with and without the antra in place. These choline esters potentiated the gastric responses to sham and real feeding, and to the local stimulation of the antrum by meat or distention. They concluded from these experiments that under physiological conditions the antrum releases gastrin at the onset of a meal by a combination of vagal excitation and local mechanical stimulations, in the presence of a low level of intragastric acidity, and that the response of the parietal cell to the circulating antral hormone is in part dependent upon concurrent cholinergic excitation. During the later course of feeding, the response of the denervated pouch after antrectomy could be potentiated by the choline esters as well. This was interpreted as

meaning that the intestinal agent(s) for stimulating gastric secretion act(s) more effectively in the presence of cholinergic stimulation, which in the intact animal would be supplied by vagal excitation.

The idea that vagotomy reduces the sensitivity of the corpus to all stimuli has become firmly accepted, although it is not in keeping with Cannon's law of supersensitivity of denervated structures. A preliminary report by Muren (45) is therefore most intriguing. Using Pavlov pouches, studied before and after vagotomy and whose acid responses to varied doses of mecholyl and histamine were established, this worker observed that while the response to histamine was not altered or even reduced, in all cases the sensitivity to mecholyl was markedly increased and the secretory response increased fourfold. This supersensitivity to mecholyl after vagotomy is indeed quite striking and one awaits the final report with considerable interest, especially information regarding the pH of the pyloric gland area of the main stomach.

*Other antral inhibitory mechanisms.*—In addition to the local acid inhibition of gastrin release from the PGA, interest also centers about the possibility of the release of another substance from the antrum which inhibits the parietal cells' activity more directly. Woodward (41) has summarized the incomplete evidence regarding inhibition of histamine stimulation of the corpus by local acid in contact with the PGA. State (46) has summarized his experiments of experimental histamine-in-beeswax ulceration in the dog in which the PGA left in continuity affords better protection than does resecting the PGA. Gillespie (47) reports evidence in man that a pH of 1.5 in the PGA markedly decreased the "maximal" histamine acid output from innervated gastric fundus, qualified by the observation that some of the acid introduced into the PGA may have exerted its effect via the duodenum. He and his colleagues (48) have recently shown that antrectomy in man reduced the "maximal" gastric acid response as well to histamine. While the lowered response of the parietal cell to histamine following vagotomy is an acceptable idea, the mechanism by which removal of the antrum has a desensitizing effect is quite obscure at present. It is of interest that Waddell & Williams (49) observed a reduction of blood flow through body and fundus in the dog after removal of PGA.

*Other gastric secretory inhibitors.*—Several substances of physiologic origin and significance have been shown to have gastric acid inhibitory powers. Several publications (50, 51) have demonstrated that glucagon inhibits the basal secretion of acid and pepsin in normal and hypersecreting patients, independent of its effects on the level of blood glucose. This holds true apparently for histamine-stimulated secretion in man at least. Papers continue to appear (52, 53) demonstrating that serotonin or its precursor 5-hydroxytryptophan also may inhibit histamine-stimulated acid secretion in men and animals. Whether these or other substances are related to the question of the unknown humoral inhibition now being sought is interesting but not resolved. Menguy & Smith (54) have shown that macromolecular material obtained from insulin-stimulated human gastric juice inhibits acid out-

put but not pepsin in the rat with ligated pylorus. The small amounts required make this animal a useful test object in the search for the mechanism of this effect, demonstrated for some time now with a variety of products of human gastric secretion.

A variety of endocrine substances depress gastric acid secretion. Nasset and his collaborators (55, 56) have shown that desiccated thyroid depressed acid secretion by an innervated dog pouch stimulated by meat, and in anesthetized rats stimulated by metacholine. Using a continuous stomach perfusion technique in rats, which have been stimulated by histamine, Ghosh has demonstrated marked inhibition by purified chorionic gonadotropin, whose gonadotrophic and inhibitory effects were not parallel. The relation of this material to urogastrone is questionable (57).

Lavers and colleagues (58) have clarified the role of thiamine deficiency in inhibiting gastric acid secretion in the dog. Degrees of deficiency which induced anorexia and peripheral neuritis inhibited insulin hypoglycemia but not the response to histamine and urecholine in vagally innervated and denervated pouches. The mechanism is presumed to be degeneration of the dorsal nucleus of the vagus.

*NH<sub>3</sub> and urease.*—Evidence summarized in the review of Kornberg & Davies (59) and rather generally accepted leads to the conclusion that the urease of the stomach which catalyzes the conversion of NH<sub>3</sub> from urea is of bacterial origin. Conway and his colleagues dispute this (60). The demonstration by Lieber & Lefevre (61) that the high gastric ammonia of patients with uremia and associated hypoacidity can be reversed temporarily by several broad spectrum antibiotics supports the generally accepted bacterial origin of this enzyme.

*Role of the liver and intestinal lymph in regulating acid secretion.*—The work of Gregory (62) clearly demonstrated that diversion of portal blood into the systemic venous system greatly augments the secretion of a denervated pouch of the stomach, both in response to sham feeding and in response to a meal. This was considered to reveal that a stimulant or stimulants of gastric secretion were released into the portal blood and that, since its release was inhibited by acid in the antrum or duodenum, the stimulant was at least in part of gastric antral origin. A finding of this work not generally appreciated was that the "maximum" secretory response of the pouch to histamine also increased, and this occurred in one dog with antrectomy so that gastrin alone is not the answer to this problem. The inference is clear that inactivation of some secretory substance by the liver normally takes place. This was also the inference of another paper (63). Silen & Eiseman (64) noted that, in dogs with portacaval shunting, meals of pure protein derivatives reproduced the hypersecretion of a mixed diet, but that pure CHO or fat diets did not reproduce this. Since the peak secretion required six hours in their experiment in contrast to the 30 minutes of Gregory, and occurred in the absence of the antrum, they related this secretory response to the intestinal rather than the gastric phase of gastric secretion, and hypothesized that histamine was the secretagogue involved. Irvine and his colleagues (65) with a different ap-

proach have reached the same conclusion that the liver normally inactivates the histamine in portal blood which was derived in their experiments from a meat meal by intestinal bacteria's decarboxylation of the L-histidine of the meal. Clarke and his colleagues (66), pursuing this further, shunted the blood drained by the superior mesenteric vein into the vena cava and induced hypersecretion which returned to normal levels after subsequent portacaval transposition. This result was interpreted as suggesting that the agents involved arise in those portions of the gut drained by the superior and inferior mesenteric veins, mainly the small bowel distal to the pancreas.

In view of the effect of the liver in inactivating gastric secretory humoral agents reaching it via portal blood, Johnston & Code (67) have sought for the presence of factors affecting gastric secretion in intestinal lymph which ordinarily bypasses the liver, and especially after a fatty meal since this is normally a potent inhibitor of gastric secretion of hydrochloric acid, and intestinal lymphatics are the major pathway of fat absorption. Their findings, while not absolutely conclusive, are most interesting. In about half the trials, specimens of lymph markedly inhibited histamine-stimulated hydrochloric acid output of denervated and innervated pouches. Mild stimulation occurred in about one quarter of trials, and no effect in the remainder. The greatest degree of inhibition occurred in lymph collected directly after a meal and was present in the nonfatty portion (cold centrifugation). The presence of systemic reactions in animals with inhibitory responses was, of course, a qualifying factor.

These experiments can perhaps be rationalized by suggesting that in the fasting state or during the vagal excitation of eating a protein meal, gastrin release is involved and that later on the intestinal release of a secretagogue normally inactivated in part by the liver plays its role. Following a fatty meal, inhibitory substances may enter the circulation via thoracic lymph. They do not shed light, however, on the importance or lack of importance of these substances in portal blood and thoracic lymph in normal digestion. While an intestinal phase of gastric secretion can be demonstrated and a gastrin-like mechanism may be involved, it is difficult to understand the role of stimulants of this phase from any adaptive or functional point of view, while the inhibition can be visualized as turning off the unneeded gastric acid secretion when the meal is in the gut.

*Duodenal mechanisms of acid inhibition.*—It has been known for a long time that acid in the duodenum (as well as in the pyloric gland area) inhibits gastric secretion and, since the work of Code & Watkinson (68), it has been generally accepted that this requires intact vagal pathways. Jones & Harkins (69) have investigated the problem anew, perfusing the isolated duodenum with hydrochloric acid, stimulating the main stomach of dogs with alcohol, and using the Heidenhain and Pavlov pouches as test organs. Inhibition occurred in both pouches, more marked in the denervated type. Their conclusion, however, that the inhibition was humoral is open to question, since it is probable that acid in the duodenum inhibited the release of gastric secretory humoral agents (gastrin  $\pm$  histamine) from the vagally

innervated main stomach by the alcohol test meal. The previous contrary conclusion of Code & Watkinson must still be accepted at present. The suggestion (70) that there is a duodenal humoral inhibitory mechanism, released from the duodenum by hydrochloric acid, which is secretin-like in character and which is effective only against gastrin stimulation, has not been confirmed since being advanced.

The mechanism by which fat placed in the duodenum inhibits gastric secretion has been approached by Menguy (71) in rats with ligated pylorus. Intraduodenal fat inhibited only when present with bile salts; apparently the absorption or action of some product of fat digestion is involved with entero-gastrone release. If the enterogastrone extracted by Gregory & Tracy (72) from the duodenum is the physiologically released hormone, it is effective against the antral mechanism only (by mechanisms unknown at present) and not effective against histamine-stimulated secretion.

#### PEPSIN SECRETION

One of the most interesting papers in a long time on pepsin secretion is that of Grossman & Marks (73) who demonstrated that the pyloric gland area of the dog that is devoid of peptic cells secretes a protease which has many of the properties of pepsinogen, without the concomitant secretion of a pepsin inhibitor. It is of interest in connection with Schofield's paper (74) to be discussed below that feeding caused a decrease in concentration of proteolytic activity. This demonstration of pepsinogen in the pyloric gland area in the dog is also of interest in connection with the recent extensive study by Taylor of gastric proteolysis (75); among other observations he has noted that on the basis of two pH maxima below five, the human stomach normally secretes two main pepsins, one of which predominates in the pyloric mucosa, and the other in the mucosa of the body and fundus. Especially interesting and somewhat speculative is his finding (76) that patients with peptic ulcer secrete an abnormal pepsin with three maxima below pH 5.

Hirschowitz and his co-workers (77, 78) have been continuing their attempts to separate the effects of secretion (release) of pepsinogen from synthesis of the proenzyme by simultaneous measurement of pepsin in gastric juice and mucosa of the rat with ligated pylorus. The results of their studies of body hydration, adrenal activity, and atropine suggest that although synthesis can proceed without release, rates of secretion can influence the rate of synthesis by some kind of negative "feedback mechanism".

Schofield, who had demonstrated in the past that the vagally denervated canine pouch secretes a relatively high basal output of pepsin, has extended his studies in an intriguing report whose full impact is not yet clear (74). The basal output of pepsin was shown to be independent of extrinsic innervation; in two dogs with high output, preganglionic sympathectomy indicated that sympathetic innervation may exert an inhibitory influence. Feeding of a small (100 to 200 gm.) or large meal (300 to 400 gm.) of meat, or meat and bread, results in inhibition of pepsin output, independent of extrinsic innervation. This inhibition of pepsin was correlated with suppression of motility



which occurred in the pouches following feeding. Since both processes were inhibited by atropine or hexamethonium it was suggested that both depend on the activity of intramural nerve plexuses. Since inhibitory mechanisms for gastric acid and motility exist in the antrum and small bowel, which may be of humoral nature, Schofield hypothesizes that a similar humoral mechanism for pepsin inhibition may also exist. (A humoral mechanism for pepsin stimulation has yet to be established, it might be added.) It is of interest in this context that Harper and colleagues (79) have shown that vago-vagal reflexes may stimulate acid and pepsin output in the cat, but this was associated with loss of tone in 80 per cent of experiments, with small contractions superimposed on loss of tonus in the half of these. With vagisection in the neck or thorax in their experiments, basal pepsin increased in one-third of the experiments, with increase in tone and motility in two-thirds of experiments. It is already clear that pepsin secretion will probably turn out to have as complex a system of regulation as acid secretion.

#### GASTRIC MUCUS

Interest in ACTH and adrenal corticoids in gastric function has lessened in relation to acid secretion. However, the paper of Clarke, Neill & Welbourn (80) deserves citation. Continued injection of corticotropin, cortisone, methyl prednisolone, and aldosterone augmented the response of denervated canine pouches to histamine and antral stimulation. Most interesting was the observation that there was a concomitant 50 per cent increase in the number of parietal cells and a 36 per cent reduction in peptic cells. The effects on viscosity and protein content of the gastric juice are not impressive. Myhre (81) confirms earlier work in the dog by experiments in the rat in which ACTH and large doses of cortisone (20 mg. per kg.) retard epithelial repair of experimental gastric defects. Kowalewski (82) has shown that the incorporation of  $S^{35}$  into mucus-producing tissues and thus in the mucus secretion of the Shay rat was markedly suppressed by cortisone treatment. From a slightly different approach Räsänen (83) has focused on another element in the gastric mucosa: ACTH and cortisone decrease the number and granulation of the mast cells of the mucosal layer in the rat. Since the mast cell contains histamine, heparin, and serotonin, the net effect of these agents on mucosal function must be more complex than heretofore considered. Hill & Code (84), pursuing their studies of histamine in the gastric mucosa, have shown that of a number of stimuli only insulin hypoglycemia increased the histamine content of the canine gastric mucosa, the rise in the fundus being greater than in the pyloric gland area.

Heatley (85) in an interesting series of experiments has studied the viscosity-raising substance in pyloric and duodenal secretions in the pig and other animals which he obtains simply by its being retained on fine sintered glass filters. This material while not homogenous has the over-all composition of a "mucoprotein". The simplicity and gentleness of handling the material by this method are noteworthy. This mucosubstance was investigated as a barrier to diffusion since the "visible" mucus barrier is presumed to protect the mucosa from acid and pepsin digestion. In ingenious experiments, this



substance had only very slight effect on the speed of diffusion of hydrochloric acid and pepsin. However, this material acting as a multilayered structure with constant addition as well as removal could act as an effective barrier.

#### PEPTIC ULCER PROBLEM

Since our thinking about peptic ulcer is today posed in terms of acid pepsin aggression versus mucosal resistance, the recent accumulation of information, some of which has been touched upon above, has only deepened the complexity of the ulcer problem. Code (86) has lucidly summarized the problem from a physiologic point of view. The work of Card & Marks relating the number of parietal cells in the stomachs of patients with duodenal and gastric ulcer and the "maximal" acid output after histamine has been well known in the U. S. during the last few years through informal presentations. It is good, therefore, to have their published data (87) which describe this relationship either as linear or curvilinear. They also relate the number of parietal cells to the mucosal thickness of the stomach and to the volume of the total acid-secreting area of the stomach. These authors are well aware that this response to the dose of histamine used is not the "maximal" secretory capacity, but rather suggest that it is the resultant of the parietal cell mass and superimposed vagal tonic influences. It is somewhat disconcerting, however, in the assessment of their undoubtedly important contribution that the responses to histamine were determined in part following gastrectomy which included the pyloric gland area, and evidence already cited suggests that the response of the fundic gland to histamine is reduced after antrectomy, although not in every subject of study. This last point, however, requires further confirmation. No significant information applying these physiologic factors to medical therapy has appeared in the period of this review. The surgical approach to ulcer disease has always attempted to apply current physiology more directly and perhaps more naively to the solution of ulcer disease by attempting to control the secretory mechanism. Surgical therapy of an investigative nature focuses therefore on the vagus mechanism and the PGA for gastrin release. Grossman (38) has succinctly summarized the complex results of vagotomy on (a) increasing gastrin release, and (b) depressing acid output from the glands with the second as the net resultant. As for the role of PGA in surgical therapy, the present climate of opinion is much more unsettled. Exclusion of the antrum from gastric continuity is clearly unjustified, a fact known clinically for a long time, now justified by physiologic experimentation. In view of the stimulating and inhibitory effects of the PGA, the question of its retention or resection poses a question not yet solved (88), since current animal experiments are not easily and directly translated to human subjects (89).

#### EXOCRINE PANCREAS

A summary of currently accepted information on the exocrine pancreas is conveniently available in a recent four-part symposium [Hokin, Jorpes, Harper, Thomas (90 to 93)].

*Fluid and electrolyte secretion.*—The site of water and electrolyte secretion

within the pancreas, a classical crux, may be a little closer to solution. Employing light microscopy and histochemical techniques for demonstrating adenosine triphosphatase and 5-nucleotidase, Wachstein & Meisel (94) demonstrated consistent straining of the so-called "secretory capillaries" within the centrally located acinar lumen and the lateral intercellular recesses which extend between neighboring acinar cells and which have a striking similarity to the bile canaliculi of the liver cell. In a beautifully illustrated paper, Ekholm & Edlund (95) have investigated the ultrastructure of the human exocrine pancreas by electronmicroscopy. They point out that the acinar lumina are bounded by acinar cells, or by acinar and centroacinar cells intermixed. Of interest in this context is the absence in the centroacinar cells of the typical system of  $\alpha$ -cytomembranes associated with zymogen secretion. In addition, the ductules, interpreted by them as the intercalated ducts, are composed of two types of cells; one resembling the centroacinar cell, but, more important, all types bear microvilli. The marked scarcity of enzyme-synthesizing structures, and the presence of microvilli on the apical cell surfaces of the intercalated ducts point to this region as one of fluid secretion or absorption. Further, recent studies using new methods for staining carbonic anhydrase appear to locate this enzyme in the intercalated duct area, with none in the acinar cell (96). All these findings bear out the early surmise of Grossman & Ivy (97) that the intercalated duct is probably the site of fluid and electrolyte formation, which was based on the alterations in threshold for secretin stimulation of fluid following alloxan damage of this area. The present reviewer derives considerable comfort from these findings since he has postulated that the effects of carbonic anhydrase inhibition of secretin-stimulated secretion may be exerted at the intercalated duct region.

As pointed out by last year's reviewer, the study of pancreatic electrolyte secretion has not received concentrated attention in recent years. The present reviewer and his colleagues (98) have presented evidence which suggested to them that the pancreas elaborates a primary or "precursor" solution of Na and  $\text{KHCO}_3$ , isotonic with the plasma, which then undergoes alteration by an exchange of pancreatic juice  $\text{HCO}_3^-$  for plasma  $\text{Cl}^-$  as the juice moves down the collecting apparatus. They postulate the intercalated duct as the site of this exchange. The normal or apparent dependence of  $\text{HCO}_3^-$  concentration on rate of flow was dissociated by acetazolamide. Further studies by this group (99) indicate that in man cholinergic blockade following secretin stimulation of water and  $\text{HCO}_3^-$  output leads to a similar dissociation of  $\text{HCO}_3^-$  and flow rate, which appears to them consistent with the concept of a primary precursor solution. The maintenance of high  $\text{H}^+$  concentration when gastric flow rates are sharply reduced by atropine presents an analogous situation. Although Nordgren & Öbrink have argued that this is in part a dead space error, they also have suggestive evidence that atropine changes gastric mucosal permeability (100). And such may be the case in the permeability of the ductular epithelium.

*Bile electrolytes and pancreatic electrolytes.*—It has been known for years

that secretin stimulates the flow of hepatic bile without influencing the output of organic solids. Although the electrolyte composition of secretin-stimulated fluid from the pancreas has been comparatively well studied, such has not been the case for the electrolyte composition of secretin-stimulated hepatic bile. Wheeler & Ramos (101) in a most interesting report, studied the electrolyte composition in unanesthetized cholecystectomized dogs fitted with Thomas duodenal cannulae. The osmolality of all bile samples was identical with plasma osmolality, despite the presence of varying amounts of taurocholate, ascribed by the authors to aggregation of taurocholate ions in micelles. Bile stored in the common duct was similar to gallbladder bile which suggests the possibility of reabsorptive mechanisms in the bile ducts. Of greater interest in the present context was the observation that when high flows were obtained with secretin,  $\text{HCO}_3^-$  concentration rose. The maximum observed was 50 m.eq. per l. The authors suggest that the hepatic bile is a mixture of a "taurocholate fraction" caused by the active secretion of bile salts, and an "electrolyte fraction" similar to pancreatic electrolyte fluid. They have calculated that at highest rates of flow this "electrolyte fraction" is roughly 75 m.eq. per l. of  $\text{HCO}_3^-$ , and an equal concentration of  $\text{Cl}^-$ , which is of course a much lower bicarbonate than that observed in pure pancreatic juice, where  $\text{HCO}_3^-$  often reaches 150 m.eq. per l. or higher. The effect of acetazolamide on bile flow and composition in their hands is strikingly different from the effects of acetazolamide (Diamox) in pancreatic fluid. Acetazolamide surprisingly enough increased bile flow:  $\text{Cl}^-$  concentration rose, and  $\text{HCO}_3^-$  fell. The net effect was an increased excretion of salt. If there is an  $\text{HCO}_3^-$ - $\text{Cl}^-$  exchange in bile ducts which some of their data suggest, although the authors do not, it would appear to be differently oriented from the one postulated for the pancreas, and the effects of the carbonic anhydrase inhibitor consequently inverted.

*Liver and secretin activity.*—Although older work had indicated that the administration of secretin by a systemic vein gave no other response than administration into the portal system, two groups of workers (102, 103) have restudied the problem with conflicting results. While the liver clearly appears to affect some gastric stimulating humoral agent(s) absorbed into the portal blood, the bulk of evidence is against similar inactivation of secretin which normally enters the portal circulation.

*Vagal innervation and pancreatic secretion.*—Studies in recent years on the role of secretin and pancreozymin in the regulation of pancreatic secretion have tended to minimize the role of the nervous system. Although a cephalic phase of pancreatic secretion probably exists in the dog, it has not been demonstrated in man, and Alphin & Lin (104) have been unable to establish its existence in the rat clearly. In addition cholinergic pathways do not seem to be involved in the release of both hormones (secretin, pancreozymin) from the upper small bowel.

*Gastric phase of pancreatic secretion.*—Older workers were unable to demonstrate a gastric phase of pancreatic secretion. Hudok & Lawrence (105)

also have failed to stimulate pancreatic fluid secretion by instilling a number of substances (fat, protein, amino acids, CHO, alcohol,  $\text{NaHCO}_3$ , and HCl) into gastric pouches or the isolated innervated stomach.

Harper has for some time been concerned with the possibility that afferent vagal fibers might be stimulated reflexly during digestion by impulses passing up the visceral afferent fibers from the abdomen, since the overwhelming number of fibers of the abdominal vagus, in the cat for example, are afferents (93). He and his colleagues (79) have demonstrated that stimulation of the distal cut end of an abdominal vagus branch resulted in reflex secretion, not only of acid and pepsin by the stomach, but also of amylase by the pancreas, although less frequently. This reflex secretory response was abolished after section of the vagus in the neck or thorax. Since some of the vagal afferents end in receptors within the gut, sensitive to chemicals or to tension, the experiments of White *et al.* (106) extend this line of inquiry further. These workers noted an increase both in volume and protein content of pancreatic juice in dogs following balloon distention of the stomach, which was abolished by bilateral thoracic vagotomy or vagal block with procaine. The existence of such reflex mechanisms for pancreatic secretion appears quite firmly established; their importance during the digestive processes still needs to be determined.

The interrelation between vagal innervation and hormonal stimulation of pancreatic secretion is further emphasized by studies (99) in which the volume and enzyme output of the human pancreas to secretin was profoundly reduced by cholinergic blockade. The relationship between pancreatic secretion and blood flow remains in dispute. Holton & Jones (107) have reported on the blood content (not blood flow) of the cat's pancreas measured by an indirect method. While acetylcholine and histamine cause changes interpreted as vasodilation, and abolished by atropine or an anti-histamine, there was no effect on secretion. Secretin and pancreozymin caused vasodilation lasting two to five minutes unaffected by atropine or antihistamines, with corresponding increases in fluid and enzyme output.

*Pancreatic enzymes.*—Marchis-Mouren *et al.* (108) have extended their studies of hog pancreatic lipase and have prepared their material so that it is homogenous by zone electrophoresis and chromatography. It appears to be a single lipase, capable of acting on tri-, di-, and monoglycerides, the reaction rate decreasing in that order. The turnover rate of 12 times the molecular weight is probably important for an enzyme acting in a heterogenous medium, since its substrate is insoluble. Some controversy has existed as to the separateness of pancreatic elastase from  $\alpha$ -chymotrypsinogen. A recent study (109) indicates that purified elastase is highly homogenous, and could be separated from trypsin and known chymotrypsins. The action of this enzyme is rather diffuse, since it has been reported to act on the B-chain of insulin.

*Dietary adaptation.*—The problem of adaptation of pancreatic secretion to variations in diet is a difficult and unresolved one. Magee & Hong (110) studied the effect of dietary factors in the daily output of pancreatic juice in

dogs. By increasing the volume and fat content of the diet the volume of juice and its proteolytic and lipolytic activity was increased. Casein hydrolysate had no effect; peptone increased volume without enzyme output. These responses are difficult to rationalize, and present some disagreement with the work of Wang & Grossman (111), especially the failure of peptone and casein hydrolysate to stimulate enzyme output, presumably by pancreaticozymine release.

*Incorporation of amino acid into pancreatic juice enzymes.*—The rapidity of synthetic processes within the pancreatic acinar cells, or the exchangeability of amino acids of these synthesized enzymes is strikingly demonstrated by the study of Hansson (112) in which labelled intravenously administered amino acids appeared within 40 to 60 minutes within the exocrine cell, at first in the microsomal fraction, later in the zymogen granule fraction. Excretion of newly produced, or newly labelled protein appeared in pancreatic juice within 45 to 60 minutes, with peak protein-bound radioactivity in one to two hours after injection.

#### PROTEIN SECRETION AND PROTEIN LOSS INTO THE GUT

The study of the macromolecules of the gastrointestinal secretions has been heavy going in recent years, stimulated mainly by the work of Glass using paper electrophoretic techniques. The multiplicity of mucoprotein components revealed by these methods has defied easy synthesis into any simple account. During the last year, however, the demonstration that the serum proteins, especially albumen, find their way into the gastrointestinal tract under several normal and abnormal conditions has been a significant advance in resolving some of the complexities of this difficult area. This has come about through several different approaches.

Gullberg & Olhagen (113) have repeated Glass' study of mixed gastric juice by paper electrophoresis, but took the simple precaution of introducing a phosphate buffer into the human stomach in an effort to minimize proteolytic digestion at acid pH's. Under these conditions three main protein components were sorted out. One migrating most rapidly towards the anode was identified as pepsin; a second with high carbohydrate content as stained by the periodic acid-Schiff reagent was a mucoprotein; and most striking was the third component, which resembled pure serum albumen, electrophoretically and immunologically. Unneutralized juice contained between two and eight components, the majority of which these authors believe are the breakdown products of proteolysis. Preliminary reports (114, 115) have confirmed the presence of serum albumen in buffered or anacid human gastric juice, and neutral canine gastric mucus, respectively. At about the same time Holman & Sleisenger (116) also reported that if precautions are taken to minimize proteolysis in intestinal juice, albumen could be recovered from the intestinal tract in normal man, and reported evidence that globulins as well could be identified in the intestinal contents. Studies of colonic mucus, reported in preliminary form, and employing immuno-electrophoresis, also have demonstrated the presence of most of the normal components of the

plasma protein in the gut (117). The general weight of this line of evidence suggests that an amount of plasma protein, still to be quantified, normally finds its way into the gastric, intestinal, and colonic secretions. The contributions of the liver and pancreas to this have yet to be established, although Katsuki and collaborators (118) have presented evidence of the presence of serum albumen and globulins in concentrated gallbladder bile. It is obvious already that the "fourth compartment" will have to be taken into account in studies of protein turnover as well as of fluid distribution throughout the body.

Paralleling and in part preceding this line of inquiry, a fascinating series of clinical investigations has uncovered the importance of abnormal losses of plasma proteins into the gut in a variety of disorders. It has been recognized for many years that the condition known as giant folds of the stomach (Menetrier's disease) may occasionally be associated with peripheral edema caused by hypoproteinemia, specifically hypoalbumenemia. Some years ago Citrin and his co-workers (119), using  $I^{131}$ -labelled albumen and electrophoretic studies of gastric contents, demonstrated that this hypoalbumenemia was caused by loss of albumen into the stomach, and sporadic case reports have followed. Great impetus, however, was given to this field by the invention of a technique by Gordon which obviates the problem of proteolytic digestion of proteins lost into the gastrointestinal tract (120). The plasma expander polyvinylpyrrolidone, labelled with  $I^{131}$  prepared by this worker, has the approximate molecular size of serum albumen, and resists enzymatic digestion. Given by vein, less than one per cent of the administered dose finds its way into the stool in three days in a normal subject. With this method Gordon has been able to unravel the hitherto unresolved problem of "idiopathic hypoproteinemia", and demonstrate that the levels of serum albumen in youngsters afflicted with this disorder can be explained by loss of albumen into the gastrointestinal tract (121). This method has quickly been applied to a number of conditions characterized by low serum albumen with success (122). In the majority of conditions so far reported—giant folds of the stomach, carcinoma of the linitis plastica type, the "exudative enteropathy" of Gordon (idiopathic hypoalbumenemia), and inflammatory disorders of the small and large bowel—structural abnormalities of the gut have been found, although in some (congestive heart failure caused by pericardial or tricuspid disease) none has yet been reported.

"Transudation" of plasma proteins into many gastrointestinal secretions has thus been fairly well established, although the route of transport is completely unknown. Since this persists throughout life, it is probably not related to the permeability of the newborn's intestinal membrane to immunologically significant proteins in the neo-natal period. Whether the "exudation" of protein in a variety of pathological conditions represents an exaggeration of the normal mechanism, or an entirely different one, remains the most challenging question in this field.



## INTESTINAL SECRETION OF OTHER SUBSTANCES

That the gut is a two-way street as far as water and electrolytes are concerned is a modern commonplace. The studies just cited reveal that the intestinal membrane is permeable to the plasma proteins. This probably holds true for other components of the body as well. Cheng & Stanley (123) have demonstrated the secretion of 400 mg. of cholesterol daily into the gut in patients with complete obstruction of the common bile duct, thus excluding the fraction of cholesterol that gains access into the gut by the bile. Whether the intravenously administered labelled fatty acid-albumen complex which Burr and his colleagues (124) found in the intestine, and which formed the bulk of the labelled lipid (11% of administered dose), was secreted solely by the intestinal mucosa remains to be determined. This and the previously cited protein studies do, however, raise interesting questions regarding the possible role of intestinal loss in so-called "mal-absorption" syndromes. Indeed Planche and his co-workers (125) have made the interesting suggestion that pancreatic lipolytic activity affects not only the absorption of dietary fats but also the lipids secreted by the intestine, as demonstrated by subjects on lipid-free diets.

## SMALL INTESTINE

The important studies by LeBlond on cell renewal in the gastrointestinal tract and beautifully documented by LeBlond & Messier (126) for the small bowel indicate that cells divide in the crypts and migrate to the tips of the villi where they are shed. Regeneration of the villi after infarction in the rat (127) occurs by outgrowth and movement of cells derived from crypt remnants. This reconstruction of the surface lining is similar to that observed in replacing the surface epithelial cells of the stomach. Quastler & Sherman (128) have attempted to rationalize the movement of cell populations in labelled cells of the small bowel by simple and reasonable assumptions regarding a steady-state system, size of compartments, and average time a cell remains in these compartments (crypts, and in movement up the villus).

*Duodenal mucosal secretion.*—The classical locus of information regarding the specialized function of the duodenal mucosa and its Brunner's glands will be Grossman's review (129). Hartiala and his co-worker Lehtiner (130) have continued their investigation of the duodenal glucuronide conjugating mechanism. A number of estrogenic steroids (estrone, estradiol, estriol, equiline, and stilbestrol) were conjugated as glucuronides, while neither progesterone nor its metabolite pregnandiol and no androgens were conjugated. The common property appears to be a phenolic hydroxyl group in the C<sub>3</sub> position as would hold true for phenolphthalein and cinchophen. The ability of the mucosa to conjugate glucuronides *in vitro* with *o*-aminophenol was markedly reduced by pretreating rats with cortisone; hepatic conjugation was unaffected [Halm, Hartiala & Pekanmaki (131)]. The role of this



mucosa in duodenal ulcer and the effects of cortisone on the disease are unknown and may possibly be related to this detoxifying mechanism in the duodenal mucosa.

Dische and his colleagues (132) have centered their studies on the mucoid fraction from duodenal glands in children and adults. Ninety per cent of the nondialyzable carbohydrate is present as trichloroacetic acid soluble mucoids containing 70 to 75 per cent CHO with the composition of a fucopolysaccharide, which is a mixture of at least two components, containing different ratios of galactose and mannose. Unfortunately this duodenal fluid is a mixture of several secretions (biliary, pancreatic, and duodenal).

*Intestinal sources of amylase.*—The amylase of the small intestine of the rat has been studied recently by two groups of investigators (133, 134). Levels of the enzyme drop with fasting, while the levels of salivariectomized-pancreatectomized animals remain unchanged, as with those fed a nonprotein diet for several days. Ordinarily the jejunal level of enzymes is higher than the duodenal, but this is reversed by fasting or liver damage. Both groups concluded that the upper small bowel synthesizes amylase. Recent studies of the effect on alteration in carbohydrate utilization and liver damage on the levels of circulating amylase tend also to restore a role to the liver in contributing to serum levels (135). While the pancreatic and salivary amylase make a contribution to serum amylase, their share is relatively small. The evidence for this has recently been summarized (136). Electrophoretic mobility studies also emphasize the heterogeneity of sources of the serum amylase(s) (137).

#### INTESTINAL ABSORPTION

The introduction of *in vitro* methods for the study of small bowel absorption by Fisher & Parsons (1949) and especially Wilson & Wiseman's description of the everted sac technique (1954) has led to an "intestinal explosion" of heroic dimensions as these techniques have been successively applied to almost every substance known to find its way from the intestinal lumen into the blood. If these methods represented a "methodological revolution", a "philosophical" revolution has been announced by Hogben (31). Current research on intestinal absorption must grapple with the thesis that the epithelial cell of the intestine is functionally similar to other cells of the body having a membrane which is essentially lipoidal in character. Thus this cell presents no special barrier to the movement of molecularly dispersed lipids, and the absorption of most drugs can be accounted for by their unionized moiety at the ambient pH of the gut. Indeed if special mechanisms are required, it is for the water-soluble lipid-insoluble solutes that they must be demonstrated.

The *in vitro* sac technique does, however, differ from the intestine *in vivo*. Chalfin *et al.* (138) have indicated that it becomes more permeable to passive diffusion of water-soluble solutes, and Parsons (139) has emphasized the difference in the sensitivity of the *in vitro* sac to inhibition of respiration by triethyltin. He has ascribed the lowered capacity of the *in vitro* sac to transport water and glucose to loss of ATP during the incubation period.

**Carbohydrate absorption.**—Monosaccharides move across two cell membranes in the gut and against concentration gradients; the details of this process have been continually explored with *in vitro* sac techniques. With sugar and sugar derivatives now totalling 49 compounds, Wilson & Landau (140) have drawn certain conclusions. Modifications about C-1 still retain in the capacity to be transported (although —S—Au substitution for —OH gives an inactive substance); modification of C<sub>2</sub> leads to inactivated compounds; OH at C<sub>3,4,6</sub> is not essential, and older work reveals that 5-0-methyl glucose is poorly absorbed.

There are limitations of the size of substituents; thus at C<sub>3</sub>—3, deoxy and 3-0-methoxy derivatives are transported, but 3-0-propyl, 3-0-butyl, and 3-0-hydroxyethyl are not. Steric orientation is important: only *d*-isomers of glucose, galactose, and deoxygalactose are transported.

The number of pathways by which monosaccharides are transported is unknown. Older and recent *in vitro* work indicates that glucose and galactose compete for a common pathway (141). Csaky & Fernald (142) report that there is probably competition between *d*-glucose and 3-methylglucose in the frog intestine. But the total evidence on this point is quite fragmentary.

The site of action of phlorhizin in inhibiting glucose absorption in the intestine has been further investigated by Newey, Parsons & Smyth (143), using a method for measuring the endogenous glucose metabolism of the intestine *in vitro*. Phlorhizin inhibited mucosal and serosal entry of glucose at concentrations which had no effect on endogenous metabolism. They concluded that phlorhizin inhibits glucose absorption by acting on a mechanism responsible for the passage of glucose across the membrane on the luminal side of the mucosal cell. They felt that their experiments did not give any evidence about the nature of the entry mechanisms which might be either a facilitated diffusion or an energy-requiring process, a reservation also stressed by Csaky & Fernald (142).

**Amino acid absorption.**—The belief that the *d*-isomers of amino acid are absorbed purely by passive diffusion has been challenged theoretically (31) and experimentally (144, 145). Paine *et al.* (144) studied the absorption of methionine and histidine in Thiry-Vella fistula in adult chickens. The L-isomers as expected were absorbed more rapidly than the *d* forms. However, the absorption of L-histidine was impaired in the presence of equimolar concentrations of L- or D-methionine, without evidence of racemization of D-methionine. These results suggest that the L-isomer of methionine and histidine is absorbed from the intestine by a common specific pathway, with D-methionine participating in one part of this transport mechanism. Similar conclusions denying the purely passive diffusion of the D form of methionine and histidine were also drawn by Jarvis & Smyth (145); in all cases the rate of absorption was not proportional to the initial concentration, revealing a relationship which corresponded approximately to Michaelis-Menten kinetics. This relationship, however, as the authors clearly stress, does not prove conclusively that either a carrier system or an enzyme system is involved. Newey & Smyth (146), using rat small intestine everted sacs, and the synthetic peptide glycylglycine, believe that the extracellular peptidases

present were capable of having hydrolyzed only a small fraction of the peptide which disappeared from the mucosal side. Yet upper mesenteric blood in the dog after several types of protein meal, and studied by a sensitive method, contained no peptides (147), confirming again the general belief that proteins enter the gut cell as amino acids, certainly leave the mucosal cell as such.

Study of the morphology of absorption so prominent now in the study of fat absorption (see below) has also been pursued in the case of protein feeding. Adamstone (148) has commented on the prominence and response of the Golgi apparatus to the ingestion of protein in the rat. However, the active role of this organelle in absorption or digestion is still obscure.

*Iron absorption.*—Using everted gut sacs of the rat, Dowdle *et al.* (149) have demonstrated transport of  $\text{Fe}^{59}$  from the mucosal to the serosal side against concentration gradient *in vitro*, and dependent on oxidative metabolism and phosphate-energy bonds. The process, maximal in the region of the small intestine immediately distal to the pylorus, diminishes in more distal segments and is accelerated by addition of ascorbic acid. One looks forward to information on the effects of body stores of iron on this process. The increased absorption of  $\text{Fe}^{59}$  by simultaneous intravenous administration of inosine in fasting rats (150) is interesting but inexplicable at present.

*Calcium absorption.*—Some of the factors regulating calcium absorption in the intact organism as reviewed by Harrison (151) have been systematically explored in the isolated proximal gut of the rat by Schachter, Dowdle & Shenker, in a series of most interesting papers (152 to 154). The active transport of  $\text{Ca}^{45}$  appears to involve two steps: (a) rapid absorption of Ca from the intestinal lumen, and (b) transfer of Ca from the mucosa to the serosal side, mainly as ionized Ca, which is specific with no accumulation in the serosal fluid of Mg, Sr, or Ba. It is greater in growing animals, and pregnant ones, and dependent on vitamin D. Active transport is increased by administration of calciferol to depleted animals. Dihydrotachysterol ( $\text{AT}_{10}$ ) was somewhat more effective than  $\text{D}_2$  or  $\text{D}_3$  although these vitamins were equally effective. Low calcium diet increased, and thyroparathyroidectomy decreased transport with vitamin D required for its demonstration. This transport mechanism is thus sensitive to the requirements of the organism and the diet proffered the gut. The initial stage of accumulation of  $\text{Ca}^{45}$  by intestinal slices was further investigated by these workers. The concentration ratio of tissue to media of five under their conditions was reduced to two by interference with oxidative-phosphorylation. Accumulation appears relatively specific for Ca, in contrast to Sr or Ba, and is dependent on dietary vitamin D. The enhancement of calcium absorption *in vivo* by lactose remains inexplicable in terms of solubilization of this ion and apparently is independent of intestinal bacterial action (155).

*Vitamin  $\text{B}_{12}$  absorption.*—Studies on the mechanism of absorption of vitamin  $\text{B}_{12}$  in isolated intestinal sacs appeared frequently during this past year. It is generally agreed that in the absence of gastric intrinsic factor (IF),  $\text{B}_{12}$  can be absorbed if present in extremely high concentrations in the

intestinal lumen, presumably by mass action or passive diffusion. In lower or physiologic concentrations, the absorption of  $B_{12}$  appears to be stepwise dependent on binding to IF and binding of IF to intestinal receptors in the presence of calcium (156 to 158). In the hamster and guinea pig, the lowest portion of the ileum was most active in  $B_{12}$  uptake in the presence of IF, with the upper jejunum showing little or no uptake, a finding consistent with current clinical opinion.

Thus special mechanisms appear to exist for the transfer of monosaccharides, amino acids, Fe, Ca,  $B_{12}$  and probably for some pyrimidines [thymine and uracil (159)]. Many more remain to be explicated.

*Fat absorption.*—The renewed interest in the morphology of absorption stimulated by the electron microscope strikes this reviewer as part of a generally healthy attempt to relate structure again to function within the gastrointestinal tract. The studies of Palay & Karlin (160) indicated that fat drops appear in the striated border of the intestinal cell between microvilli; in the studies of Wotton & Hairstone (161) the fat droplet appeared within the microvilli. In both studies the implication is clear enough that fat is absorbed in the form of large aggregates. Last year's reviewer has vigorously presented his rejection of the concept of aggregate absorption of fat, with or without the process of pinocytosis. The electronmicroscopic studies certainly do not allow conclusions to be drawn as yet as to activities occurring at the cell membrane. Juhlin, however, has reported studies (162) with solid spherical particles measuring  $0.01\ \mu$  to  $1.2\ \mu$  fed to rats and mice; both positive and negatively charged particles were not absorbed through the intestinal mucosa, although there are significant differences between solid micro-particles and deformable microdrops. Adamstone (163) has found a marked increase in the Golgi apparatus counts of animals fed fats, as well as protein as noted above, whose significance is as obscure as in protein absorption.

Despite the classical studies of Borgström and his collaborators (164) that fat absorption is virtually completed in the first 100 cms. of the upper small bowel, attempts continue to be made clinically to implicate disorders of the ileum in steatorrhea. Whether or not it is assumed that fatty acids are absorbed by active transport, or as the result of local pH changes dependent on electrolyte transport, studies in inverted sacs of hamsters (165) and homogenates of rat and human gut (166) indicate that the proximal jejunum is the preferential site of fatty acid incorporation into triglyceride of the mucosa. Shepherd & Simmonds (167) have attempted to quantitate the limits of fat absorption from the duodenum by measuring lymph fat outflow during intraduodenal fat infusion. Using coconut oil, a steady state was achieved when 24–48 gm./gm./100 gm. body weight were infused into the duodenum per hour. An interesting sidelight was the fact that the fat output was enhanced by increased intraduodenal fluid input.

The studies of Dawson & Isselbacher (166) on the esterification of palmitate- $1-C^{14}$  by homogenates of rat and human mucosa are especially interesting. The system is dependent upon coenzyme A, ATP, and Mg but does not incorporate short-chain fatty acids. The neutral labelled fat formed was

a mixture of mono-, di-, and triglycerides. Extending their studies to slices of small intestine *in vitro*, these authors (168) have stressed the role of bile salts on the process of esterification just described. When palmitate-1- $C^{14}$  is incubated with gut and buffer, binding to the cell surface takes place but no significant esterification. Binding is also facilitated by wetting agents such as "Tween 80". The addition of conjugated bile salts stimulates esterification, which is ascribed to an effect on mucosal cell metabolism, beyond their effect on fatty acid solubility, since they stimulate incorporation of  $C^{14}$  derived from glucose into mucosal lipid. The free salts behave differently from the conjugated derivatives. Both cholate and deoxycholate inhibit glucose transport by the small bowel and cause histologic damage. Deoxycholate inhibits esterification, and cholate stimulates only slightly. This inhibiting effect of free bile salts has recently been demonstrated by Menguy on acid secretion also (169).

**Cholesterol absorption.**—The role of bile salts in promoting entry of cholesterol as well into the mucosal cell, described earlier by Swell and associates (170), after administration of taurocholate, has been extended by Vahouny, Gregorian & Treadwell (171) who observed comparable significant rises in total lymph cholesterol following either glycocholate or taurocholate. Cholic acid (with 3 free hydroxyl groups and an unconjugated carboxyl group) was the most effective bile acid in facilitating cholesterol absorption.

Swell, Field & Treadwell (172) have presented further evidence based on the absorption of cholesterol-4- $C^{14}$  oleate, and cholesterol-4- $C^{14}$  that only free cholesterol can enter the intestinal wall. The studies of Gordon & Cekleniak (173) on the absorption of the methyl ether of cholesterol, which could not be esterified, would also indicate that esterification is not a prerequisite for intestinal absorption. Swell, Trout, Field & Treadwell (174) interpret their data on increases of lipid in mucosal and lymph cholesterol, when taurocholate and oleic acid are given with tracer dose of cholesterol- $C^{14}$ , to mean that the cholesterol entering from the lumen is mixed with a pool of free cholesterol in the mucosa before incorporation into chylomicrons. Cholesterol fed as the free form, acetate, or palmitate (175), when determined in mucosal cells and their subcellular components, was found predominantly as the free compound and in the microsomal fraction of the mucosal cell. Dihydrocholesterol is absorbed less efficiently than cholesterol from mixtures, when determined in rat lymph, but like cholesterol is found predominantly esterified in thoracic lymph (176).

The reorientation in thinking presupposed by Hogben's unifying concept of the intestinal cell as one which presents no special barrier to the movement of molecularly dispersed lipids raises, as its author is well aware, other problems. Some drag would be required to move luminal lipid by passive diffusion into the luminal cell, and Hogben has placed this "sink" (in his term) in the aggregation of lipids in chylomicrons in the intestinal lacteals.

**Water and electrolyte movements.**—Water absorption requires the presence of glucose in the jejunal fluid and can be absorbed against an appreciable gradient of water. Barry, Matthews & Smyth (177) observed water and glucose movements in everted rat sacs, dividing the small bowel into five equal

parts. Transfer of glucose and water was greatest in the middle sac (III) and least in sac V, without strict correlation between the two. The lower end of the ileum had little capacity for glucose transfer but considerable capacity for water, and phlorhizin consequently reduced fluid transfer in sac I and III but had little effect on sac V.

It is held that the potential difference in the isolated intestine is developed by active  $\text{Na}^+$  transport, and that  $\text{Cl}^-$  is transferred by passive diffusion and exchange diffusion, presumably with  $\text{HCO}_3^-$ . One awaits, therefore, the publication of data which led Tidball (178) to conclude that chloride is actively transported, in contrast to additional evidence by Clarkson & Rothstein (179) that only sodium among monovalent ions moves against electrochemical gradients in the small bowel.

The situation in the colon is different as regards water and electrolyte absorption. Cooperstein & Brockman (180) attributed fluid movement to active transport of  $\text{Na}^+$ , with  $\text{Cl}^-$  following passively. Curran & Schwartz (181) have confirmed this in essence in the colon of anesthetized rats, perfused *in vivo*. Water transport was found to depend entirely on the rate of  $\text{Na}^+$  transport, and was entirely passive. Sodium absorption occurred against electrochemical gradients and is interpreted as evidence of active transport. Chloride movement was found to be caused almost entirely by passive diffusion.

### MUSCULAR ACTIVITY

The focus of current interest in esophageal motility continues to be concerned with the closing mechanism of the lower end of the esophagus. The role of the high-pressure zone and ancillary mechanisms has been amply reviewed by Vantrappen, Texter, Baborka & Vandenbroncke (182). Abnormal nonperistaltic motor activity, i.e., rhythmic esophageal pressure waves in the absence of a normal deglutition pressure wave across three closely related recording sites, was induced by intraesophageal installation of 0.1 N HCl in humans and coincided with "heartburn" (183). Schenk & Frederickson (184), investigating cardiac and cricopharyngeal sphincter thresholds in the cat, assigned the major role to intrinsic smooth muscle mechanisms, yet after section of the diaphragmatic attachment, esophageal sphincter thresholds decreased approximately one-third.

Feeding responses of the small bowel are well known and have been observed in exteriorized loops and after sham feeding. Stickney & Northup (185), investigating the effects of gastric emptying upon the propulsive motility of the rat's small intestine, found that the latter was independent of the stomach, although osmolar receptors in the upper duodenum appear to retard gastric emptying (2).

Reference has already been made to the experiments of Harper *et al.* (79) on the effects of stimulation of the central cut end of an abdominal branch of the vagus in the cat on gastric and pancreatic secretion. Reflex changes in gastric and intestinal motility also were induced by this maneuver. In 80 per cent of experiments, there was loss of gastric tone, with superimposed small contractions in about half. In the remaining 20 per cent, small contrac-



tions occurred without the fall in tone. Contractions of the small bowel were produced by either afferent or efferent vagal stimulation. Blair and his colleagues (186) have observed that the motor responses of the stomach and small bowel to slow efferent vagus stimulation are potentiated by a preceding period of fast stimulation, believed to occur in Auerbach's plexus. Choline chloride also reproduced this in some instances.

Douglas (187) has summarized older and more recent literature which indicates that the intestine is continually influenced by a rhythmic electrical excitatory process conducted at 17 to 19 pulses per min., whose "pace maker" appears to be in the duodenum. The source of the membrane potential of intestinal smooth muscle remains obscure. Barr (188), using the inulin space of distribution, believes that the potassium gradient is large enough to support the hypothesis that the resting membrane potential is essentially a diffusion potential dominated by the K gradient. Hurwitz (189) studied unidirectional fluxes of  $K^{42}$  in isolated guinea pig ileum. The addition of  $7.5 \times 10^{-6} M$  pilocarpine, which produced maximal contraction of longitudinal smooth muscle, increased K efflux 100 to 150 per cent, whereas K influx was markedly impeded. Cocaine blocked this pilocarpine-stimulated efflux, but had no effect on unstimulated efflux. Hurwitz interprets his data to suggest that the movement of potassium in excited smooth muscle fibers reflects to a large extent a permeability change attributable either to sodium or other ions which exert a depolarizing influence on the cell membrane.

Daniel and his co-workers have further (190, 191) attempted to relate electrical phenomena and contraction. In their first study, using monopolar electrodes in the dog small intestine exposed at laparotomy under anesthesia, they demonstrated the fast action potentials and slow waves seen by others. The action potentials were associated with forceful segmental contractions, regardless of the time relationship of the action potentials to the slow waves. The same situation held true for the human intestine. The rate of slow wave activity was greater in the duodenum, and the duodenal pace-maker mentioned above which has been reported to control the slow wave rate was shown to be limited to the upper intestine. In the more recent study (191) the electrical activity was recorded by microelectrodes, and periodic slow depolarization waves were observed. Anoxia diminished the frequency and amplitude of this slow depolarization without affecting the resting potential. Action potential spikes arose from the large slow depolarization. Comparing these results with the previous records of monopolar extracellular recording devices, Daniel and his associates concluded that the slow waves recorded above arise from slow depolarization of intestinal muscle cells, which may be a co-ordinating mechanism for motility of longitudinal muscle. Burnstock & Prosser (192), using the "sucrose gap" electrode in cat intestinal muscle, have demonstrated prolonged depolarization with 0.1 per cent barium chloride, or  $10^{-6}$  acetylcholine, but were unable to decide whether this was caused by maintained sodium conductance, delayed potassium conductance, or a combination of both.



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# LIVER<sup>1</sup>

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The material chosen for inclusion in this review has been arbitrarily selected in the fields of interest to the reviewer. Because of the limit of time included, there has been no attempt to indicate priority of the articles cited or to include all of the publications concerning any single subject.

## HEPATIC STRUCTURE AND FUNCTION

The functional unit of the liver is described as an acinar structure which may be simple or compound or may occur as aggregates of acini (1). Because of the fissures of the liver in animals, the vascular divisions of the portal and hepatic systems branch off in the form of two parallel fans. In the compact liver of man, both systems overlap so that each suprahepatic vein collects blood from two neighboring portal regions and the liver possesses five regions and six segments furnished with afferent and efferent pedicles (2, 3). Microangiography of rabbit livers (4) shows that the branches of the portal vein come into the lobules and give rise to sinusoid bundles or divide into two or three venules before they resolve into capillaries. The great venous plexus surrounding the biliary ducts is derived from the portal vein and constitutes a portal system placed at the wall of the intrahepatic biliary pathways before the sinusoidal system. The hepatic artery gives branches to the walls of the biliary ducts, forming a capillary network, and also gives branches to the lobules, these branches opening into the peripheral and central sinusoids. The hepatic veins alternate with portal veins. No direct communications were observed between the branches of the portal vein and hepatic vein except through the numerous sinusoids. After administration of epinephrine, the central vein is empty and marked dilatation occurs in the central sinusoids. The venous plexus of the biliary ducts is constricted. Acetylcholine produces vasodilation of the hepatic vascular system. In other animals, and in the normal human being, there are no nonsinusoidal anastomoses between the portal vein and the hepatic vein or between the hepatic artery and the portal vein (5). With cirrhosis or cancer of the liver there are many shunts from the portal to the hepatic vein and from the hepatic artery to the portal vein. A summary of present concepts of the control of sinusoidal sphincters and blood flow through the liver suggests that portal-caval anastomoses do occur in normal animals and men (6).

Good illustrations of rat liver as viewed with the electron microscope (7) show that the endothelial lining of hepatic sinusoids is predominantly continuous. The small gaps frequently seen are often near a semiattached

<sup>1</sup> The survey of literature pertaining to this review was concluded in June 1960.

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endothelial cell which seems to be in the process of closing a temporary gap. The perisinusoidal space is kept open by the hepatic microvilli and the intralobular reticular fibers. The variations in size of the reticular fibers suggest a continuous formation of new reticular fibers. The perisinusoidal space does not have a demonstrable basement membrane, but contains a continuous ground substance which may be equivalent to a basement membrane. The hepatic microvilli may have free endings or be attached to the endothelial lining. Some substances could be thus taken up by the hepatic cell directly from the blood stream while others could pass into the perisinusoidal space (8). The structure of human liver is similar to that found in animals (9). From a study of the ability to take up thorium dioxide it was found that the endothelial cells of the sinuses, Zimmermann endothelial cells, star-shaped cells, and Kupffer cells were all similar (10).

Fractionation by differential centrifugation combined with chemical procedures, histochemical methods, and examination with the electron microscope is yielding information pertinent to hepatocellular structure and function. The lysosome fraction of rat liver contains the dense bodies seen in the parenchymatous liver cells in electron micrographs. These contain considerable amounts of ferritin (11) and increase in number after administration of iron (12).

The microsomes, which are relatively rare in normal cells, are more numerous in regenerating liver and have been regarded as possible precursors of mitochondria (13). With freeze-dried preparations, these appear to be small areas of great concentration of substances which fade gradually into the surrounding cytoplasm (14). The mitochondrial count in livers of fasting mice increases for the first 48 hours and subsequently decreases (15), probably because of clumping and some increased fragility. There is an intranuclear change in the hepatic cells of rats after a short period of tryptophan deficiency (16). The intranuclear inclusions found in enlarged liver cells of mice and in mouse hepatoma cells appear to be formed by invaginations of the nuclear envelope (17). These inclusions have been found in a wide variety of conditions and were shown to contain glycogen, sudanophilic lipids, iron-containing pigments, acid and alkaline phosphatase, esterase, and  $\beta$ -glucuronidase (18).

#### SECRETION OF BILE

Good discussions of the mechanisms of biliary secretion have appeared (19, 20, 21). Each of these points out that the secretion of bile is a complicated process which involves accumulation of substances in the hepatic cells, some chemical conversion, some active secretion, and various other selective factors. The volume of bile secreted is remarkably constant under a wide range of blood pressure and blood flow and is not altered nor is its composition altered by portal-caval shunt or by arterial shunt to the portal vein, which may increase the blood flow through the liver to more than twice its normal flow (22). Hypothermia of the liver (23) reduces the volume of bile

flow in proportion to the reduction of temperature, but sodium cholate or dehydrocholate will increase the rate of bile flow in the same proportion in the cooled liver as at normal temperatures. With cooling there is a shift of extracellular fluid to intracellular compartments and a decrease in the solid contents of the bile. This appears to be in keeping with a decrease in the efficiency of transfer of materials from the blood to the parenchyma of the liver, since with sulfobromophthalein the extraction rate is reduced by a factor of 1.9 for each 10°C. fall in temperature. Chemical studies of the role of oxidative phosphorylation and the electron transport system (24, 25) on the rapid uptake of fluid by mitochondria of the liver may shed light on this process. The inhibition of bile secretion from the perfused liver by moniodoacetate (26), but not by sodium fluoride (27) and mercurial diuretics (28), and the slight depression by cyanide (29, 30), suggest that the energy for secretion is derived from metabolism of glucose.

Some of the components of bile are derived directly from the blood and appear in the bile in concentrations not differing materially from those in the blood. This has been shown for water, sodium chloride, and potassium (31). Other substances such as phosphates, alkaline phosphatase, cholesterol, phospholipids, plasma proteins, sucrose, and inulin appear in the bile in concentrations much below those in the plasma. This seems good evidence of an active secretory process and not simple filtration for the substances that appear in the bile. Other substances apparently secreted directly into the bile from the hepatic cell are in concentrations up to 1000 times those found in the blood. Many of these substances are conjugates such as taurocholates, glycocholates, bilirubin conjugates, thyronines, pressor amines, steroid hormones, and sulfobromophthalein; but unconjugated substances such as fluorescein, rose bengal, and indocyanine green (32) are also excreted directly from the hepatic cell.

The transfer of substances like sulfobromophthalein from the blood to the bile is not a simple process. Even at low rates of administration, much of the dye is stored in the liver cell and is available for later secretion. Increasing the infusion rate increases the total excretion in the bile, but the proportion of dye excreted to that administered decreases until the maximal excretion rate is equaled or exceeded by the injection rate. Other dyes, bilirubin, and bile salts exhibit this same saturation phenomenon and also interfere with the excretion of each other because of competition for uptake and excretion by the liver. The rate of excretion is not maintained until the liver is cleared following cessation of administration, but decreases rapidly and persists for considerable periods. If storage is made with labeled dye and followed by injections of unlabeled dye, the biliary excretion is composed of the freshly delivered dye and stored dye at the same rate as for the stored dye, as if no further injection had been made. From this it is apparent that absorption and excretion are separate though related processes. This viewpoint is further substantiated by the demonstration of the rate of excretion of the metabolites of sulfobromophthalein (33) and the fact that no difference is shown in



and pigment II. The diazotized products of each behaved differently on columns eluted with butanol-water at pH 4. A single slow-moving band was obtained from bilirubin and a single rapid-moving band was obtained from pigment II, while pigment I gave two bands of equal proportions, one moving like the bilirubin products and the other like the pigment II products. Absorption chromatographs on alumina, silica gel, and paper had also suggested differences in the pigment. It was also shown, by Kawai and by Billing, that two different azo pigments were formed from bile pigments reacting with diazobenzenesulfuric acid. Three independent investigators in 1956 demonstrated that bilirubin was excreted mainly as a conjugate of glucuronic acid [Billing & Lathe (64); Schmid (65); and Talafant (66)]. The azo pigment formed from bilirubin is the diazonium salt of neoxanthobilirubinic acid and isoneoxanthobilirubinic acid (67). Studies of the rapid-moving azo pigment with  $\beta$ -glucuronidase, mild alkaline hydrolysis, and glucuronic acid determinations indicated that one molecule of this pigment was equivalent to one molecule of the slow-moving pigment from bilirubin conjugated with one molecule of glucuronic acid (67, 68) and that the pigment II from bile, blood, or urine was bilirubin diglucuronide with an ester linkage. The liberation of glucuronic acid from the azo pigment by hydroxylamine is further proof of the ester linkage (69). Alkaline hydrolysis of direct bilirubin isolated by electrophoresis yielded two molecules of glucuronic acid for each molecule of bilirubin (70). Since pigment I formed both azo pigments with the van den Bergh reagent, and glucuronic acid was about molecularly equal to the bilirubin, it was suggested that pigment I was probably a monoglucuronide of bilirubin. However, this pigment has not as yet been well characterized and the presence of other glucuronides in the preparation has not been ruled out.

Bilirubin may also be conjugated with other substances. It has been esterified with taurine and found to be very similar in its reactions to the corresponding monoglucuronides and diglucuronides of bilirubin (71). Synthetic bilirubin sulfate reacts "direct" with the van den Bergh reagent (72). Bilirubin sulfate has been identified in the bile of man, cats, and rats following injections of radioactive sulfates. Chemical studies of this material indicate that the sulfate is linked to the hydroxyl groups of bilirubin rather than the carboxyl groups as is the case with glucuronide bilirubin. Human bile contains about 24 per cent of its bile pigment in a form nonhydrolyzable with glucuronidase, and 14 per cent as sulfate conjugates. About 10 per cent is acid labile and contains neither glucuronic acid nor sulfate (73, 74). An ester form of bilirubin that is alkali labile and does not contain glucuronic acid has been isolated from dog bile and also from icteric urine (75, 76).

Bilirubin glucuronide can be synthesized in the liver of man, rat, mouse, and guinea pig by the enzymic transfer of glucuronic acid from uridine diphosphoglucuronic acid to bilirubin (77 to 80) and other substances (81). Glucuronyl transferase was localized in the hepatic microsomes and found to be limited in its activity by the amount of uridine diphosphoglucuronic

acid present (79). The rat kidney was also shown to conjugate bilirubin with this same system (80). Other glucuronides may be synthesized in liver homogenates from a glucuronic acid-1-phosphate (81), and bilirubin glucuronide may be formed from uridine diphosphoglucose or from uridine triphosphate and glucose-1-phosphate if diphosphopyridine nucleotide is also present (82).

The formation of bilirubin diglucuronide takes place only in the liver and is excreted in this form in bile. The diglucuronide represents about 75 per cent of the bilirubin pigments (74, 83) but varies some in different species. In the dehepatized dog no diglucuronide bilirubin accumulates in the blood, tissues, or urine, but other conjugates (pigment I) accumulate in the blood and are found in the urine, and free bilirubin accumulates in the blood and tissues. The presence or absence of the kidneys after removal of the liver did not alter the types of pigments found in the blood. In the dehepatized rat with kidneys removed, the pigments in the blood were similar to those of the dehepatized dog. If the kidneys were present in the dehepatized rat, small amounts of bilirubin diglucuronide appeared in the blood and urine (83, 84). Since only a trace of bilirubin is present in the blood of normal rats, it is doubtful whether the kidney is much of a factor in normal metabolism of bilirubin. Bilirubin infused into rats at rates exceeding the excretory capacity of the liver produced accumulation of bilirubin and pigment I in the blood, but the diglucuronide of bilirubin did not appear in the blood (85).

When jaundice is produced in animals by the administration of hepatotoxic agents (carbon tetrachloride, toluenediamine, ethionine, or icterogenin), a large amount of the bile pigment of the blood is retained as pigment I. Some retention of bilirubin diglucuronide and free bilirubin also usually occurs (83, 84). When jaundice was produced in animals by occlusion of the biliary ducts, the bile pigments retained in the blood were more similar to those of bile, with the majority of the pigment appearing as bilirubin diglucuronide. After three to five weeks of biliary obstruction in dogs, pigment I became the chief pigment of the serum. In a clinical investigation of patients with extrahepatic obstructive jaundice, the dominant direct-reacting pigment of the serum was bilirubin diglucuronide in 88 per cent of the patients, while in a comparable group of patients suffering from jaundice of hepatocellular disease, the predominant pigment of the serum was pigment I in 84 per cent (84). Schachter, using a different method, had similar conclusions (86).

Neonatal jaundice has been shown to be associated with a deficiency of the glucuronide conjugating systems of the liver (77, 78). Homogenates of fetal liver from the rat failed to synthesize bilirubin conjugates because of a deficiency of both glucuronyl transferase and uridine diphosphoglucuronic acid (87). The conjugating system was absent at birth, but developed in the first few days until the usual adult level was found at five weeks of age. A similar, but persisting, deficiency has been shown for the Gunn strain of rats, which manifest nonhemolytic hyperbilirubinemia and fail to conjugate

bilirubin glucuronide (87, 88). Glucuronyl transferase activity and uridine diphosphoglucuronic acid are not decreased during the rapid growth period of the liver after partial hepatectomy (89). The lack of conjugating mechanisms of the fetal liver does not seem to arise from lack of substrate to stimulate enzyme formation, as sodium anthralate given a guinea pig mother and present in the fetus the last six days of gestation does not increase glucuronide formation. Bilirubin injected into the placental artery or the maternal circulation is transferred to the fetus, but the placenta seems a barrier for conjugated bilirubin so injected (90, 91). Bilirubin does not pass readily into the brain or spinal fluid of rabbits; it does so after intracisternal injection of *p*-chloromercuribenzoate, but not after anoxia or administration of menadione sodium bisulfite or sulfoxazole (92).

The placenta of Gunn rats, however, seems to be impermeable to bilirubin. Newborn rats with the inherited defect, even when born to bilirubinemic mothers, are not born jaundiced and have low levels of serum bilirubin at birth, but the levels rise rapidly in the first few hours of life. Development of kernicterus in a large percentage of the young has been reported. Typical symptoms developed, and characteristic yellow staining of the ganglion cells of the thalamus and hippocampus was found. The disease almost always developed within the first month of life, and the frequency and severity of the abnormalities of the nervous system were related to the degree of hyperbilirubinemia with some variation as to susceptibility of different animals. The jaundiced young rats were much more susceptible to sodium sulfadiazine, sodium sulfoxazole, and sulfoxazole diethanolamine than were unjaundiced rats. The serum bilirubin was reduced by these drugs, but the yellow staining of the tissues was more intense (93). An epidemic of kernicterus among premature infants treated with sulfoxazole diethanolamine has been reported (94), and it has also been reported that salicylate and sulfoxazole uncouple protein-bound bilirubin (95). Subcutaneous injections of bilirubin did not seem to increase the severity of the neurotoxic signs or the degree of central-nervous-system staining in the jaundiced rats. Exposure to hypoxia for the first seven days of life did not seem to alter the incidence or severity of the kernicterus. Intraperitoneal injections of sodium glucuronate usually produced a fall in the level of bilirubin in the young, but not in the old, jaundiced rats. No change in the incidence or severity of kernicterus was found. A similar decrease of the serum bilirubin after glucuronate injection has been observed in the jaundiced newborn (96). However, it has been shown that sodium glucuronate or glucuronolactone labeled with  $C^{14}$  appeared in the bile of dogs in only trace amounts as bilirubin glucuronide. Glucose- $C^{14}$  appeared to be a much more efficient precursor than glucuronate or glucuronolactone (97).

A deficiency of glucuronyl transferase, but normal uridine diphosphoglucuronide, has been found in the livers of patients with constitutional hepatic dysfunction, and the ability to form *o*-aminophenol glucuronide was also impaired. The retention of unconjugated bilirubin in the blood was con-

sidered to be caused by the impaired ability of the liver to conjugate and remove bilirubin although bilirubin conjugates were found in the bile (98). There are probably varying degrees of involvement in this disease, as others have been shown to conjugate *N*-acetyl-*p*-aminophenol with glucuronide (99), and salicylamide (100). In congenital jaundice of the Crigler-Najjar type with high levels of unconjugated bilirubin in the serum of children, it was found that the bile was colorless and contained no glucuronide conjugates. The ability of these children to conjugate menthol, salicylic acid, and hydrocortisone metabolites as glucuronides was also markedly impaired (101). Other types of familial nonhemolytic jaundice have been described in which there are no demonstrable lesions in the liver or biliary system to account for the retention of both unconjugated and conjugated bilirubin in the blood and tissues (102); also, recurrent idiopathic jaundice (103 to 105) and recurrent jaundice associated with pregnancy (106) have been described. Another form has been attributed to increased bilirubin formation probably associated with increased heme or its tetrapyrrole antecedents, since the survival time of the red cells was within normal limits (107). Some suggestions as to the mechanisms involved in these types of jaundice may be forthcoming from studies of icterogenin. Intraperitoneal injection of this drug in rabbits produces a colorless bile and suppression of excretion of bilirubin, sulfobromophthalein, and phyloerythryn with no histologic abnormality detectable in the liver. It is considered that icterogenin alters the permeability of the hepatic cell so that the bile is reabsorbed and passes through the liver cell into the sinusoids (108).

#### SULFOBROMOPHTHALEIN

Sulfobromophthalein has also been found to be rapidly conjugated before being excreted in the bile. Elution from bile on alumina with acetone solutions showed that in rat bile, only about 10 per cent of the sulfobromophthalein was excreted unchanged, while the remainder appeared as compounds II and III, which were of sulfobromophthalein color in alkali and were considered as conjugates. In other species some other conjugates appeared in the bile, differing in each, so that at least five different biologic conjugations may occur. None were found to be glucuronides (109). The conjugation was almost eliminated by hepatectomy in the dog (110) and in the rat (111). Separation of the metabolites in the bile and urine of normal persons and in those with various diseases of the liver also suggested the liver as the main site of conjugation in man (112). Patients with obstructive jaundice retained the conjugated dye in the serum while patients with hepatocellular injury retained the unconjugated dye. Most of the dye in the urine was conjugated, and little difference was noted in different diseases of the liver (111, 113). The dye is not excreted in human bile as a glucuronide (114). About 20 per cent of injected sulfobromophthalein is excreted as compound III, which has been characterized by hydrolysis as being conjugated with glycine and glutamic acid (115). Ninhydrin-positive material has been found with the hydrolysis

of the conjugates from bile (116), but similar treatment of control bile without sulfobromophthalein also yielded glycine and glutamic acid, and cysteine was found liberated from the conjugate which is considered a mercaptide with cysteine or the peptide glutathione (117). The clearance of sulfobromophthalein is only about half as rapid in infants one to three days old as that found a few days later (118), as is the glucuronide conjugation of acetanilid products (119).

#### CONJUGATION OF HORMONES

The liver plays an important role in the regulation of the adrenocortical steroids in the blood (120). The normal liver reduces the ketone group of the ketosteroids to form 17-hydroxycorticoids, which are conjugated with glucuronic or sulfuric acid and are excreted by the kidney as such conjugates. In the presence of severe hepatocellular disease, the reductive reaction is impaired so that administered cortisol disappears more slowly from the blood even though there is adequate conjugation of administered 17-hydroxycorticoids (121). In congenital nonhemolytic nonobstructive jaundice (122), the conjugation of 17-hydroxycorticoids, as well as the glucuronic acid conjugation of bilirubin and acetylamino-phenol, is impaired. Hydrocortisone had no effect on the volume of T-tube bile or the total excretion of bilirubin (123). Treatment of newborn rats with cortisone did not increase the glucuronyl transferase activity or the uridine diphosphoglucuronic acid content of the liver, did not increase synthesis of bilirubin glucuronide (124) or alter formation of *o*-aminophenol glucuronide (125). Steroid and other hormones have been shown to influence specifically the liver content of several pyridine nucleotide-linked dehydrogenases (126). The perfused rabbit liver rapidly forms several other 17-ketosteroids from androsterone, but does not form dehydroisoandrosterone or eticholanotone (127). Administration of adrenal steroids or ACTH does not increase the hepatic capacity for inactivation of steroids (128). Hyperaldosteronism is a major factor in the sodium retention that occurs in cirrhotics with ascites. The increased amounts of aldosterone in the urine may be caused by the failure of the liver to inactivate aldosterone, but there is also a failure of the normal mechanisms that inhibit the adrenocortical secretion of excessive aldosterone (129). On the other hand, there is thought to be some decrease in adrenal cortical function in severe disease of the liver (130).

The regulation of the amount of thyroid hormones in the blood is a function of the liver. After complete removal of the liver in the dog, administration of either  $^{131}$ I-thyroxine or tri-iodothyronine produces much less inorganic iodide than it does in intact dogs. Conjugation of these thyronines occurred without the liver and were identified in urine, blood, and tissues as glucuronides and sulfates (131). The molecular arrangement of the iodine-containing thyronines may be altered in the absence of the liver (132). About 20 per cent of thyroxine injected intravenously in dogs is excreted in the bile, mostly as conjugates of thyroxine and some newly formed thyronines con-

taining iodine (133). Somewhat more of injected tri-iodothyronine appears in the bile as glucuronide and sulfate conjugates (134, 135). About one-third of the iodine from these injected thyronines appears in the urine in 24 hours as inorganic iodide with only small difference whether or not they are removed by the biliary fistula. If the common bile duct is obstructed considerably, more inorganic iodide and thyronine conjugates appear in the urine. The compounds formed from thyroxine or tri-iodothyronine in the absence of the liver appear to be the same, but in different proportions from those in the normal animal (136). After removal of the intestines as well as the liver, the conjugated products are the same as in hepatectomized dogs (137). There is also evidence that damage to the liver slows turnover of thyroxine and that the calorogenic action is less when damage of the liver is present (138). After carbon tetrachloride poisoning in mice, the uptake of  $I^{131}$  by the liver is greater than before poisoning and the thyroid uptake is much reduced (139), but after administration of thyroid-stimulating hormone these animals take up  $I^{131}$  in a normal manner (140).

#### PORTAL BLOOD FLOW

There was little correlation between portal pressure and blood flow in the portal vein, as measured by the square-wave electromagnetic flowmeter in dogs. Splenectomy reduced the flow 38 per cent, and hemorrhagic hypotension reduced portal flow which was increased again with the replacement of blood volume (141). In unanesthetized dogs, histamine increased portal flow and pressure, acetylcholine increased blood flow with no alteration in portal pressure, while noradrenaline increased the flow and decreased the pressure, and vasopressin decreased both flow and pressure (142, 143). By the radioactive chromic phosphate method the rate of blood flow through the normal human liver was calculated as 0.9 ml. for each gram of liver each minute (144). Portal pressure has been shown to increase with increased intraperitoneal pressure (145), and this was reflected in the corresponding increase in wedged hepatic-vein pressures (146). In cirrhotic persons the gradient between hepatic-vein wedged pressure and hepatic-vein pressure was much greater than normal, and in several instances the feeding of meat produced a marked increase in hepatic-vein wedged pressure which was not found after feeding glucose (147). In both man (148) and experimental animals (149) there is a wide range of variation of portal pressure so that the term "portal hypertension" is ambiguous. Acute ligation of the portal vein of dogs is followed by a loss of 54 per cent of the extrasplanchnic circulating blood volume (150), but if portal drainage is provided and the portal blood is returned to the circulation the procedure is not fatal (151).

Hypoxia for 2 hr. reduced the acid-soluble phosphorus content of all tissues of rats, with return to normal in all tissues except the liver after 15 min. of exposure to oxygen (152). Changes in the form of the mitochondria and depression of oxidative and phosphorylative metabolism occurred in rat liver deprived of circulation. These changes were reversible after up to 90 min. of

anoxia (153). Perfusion studies indicated that the survival time of liver cells was at best only 3 hr. with deficient oxygenation (154), and changes in the electron microscopic picture have been shown with this period of hypoxia (155). Decrease of the oxygen of the blood of rabbits to 40 per cent for one hour changes the glycogen and amino acid content of the liver, and there is a marked increase in the free amino acid content of the blood during recovery, the increase continuing for several hours (156). Increased retention of carbon dioxide in the presence of adequate oxygen and with no change in pH or blood flow produces retention of sulfobromophthalein in dogs (157). Low rates of hepatic blood flow may also reduce clearance of sulfobromophthalein (158). Hypothermia reduces hepatic blood flow, oxygen consumption, and biliary secretion, but the changes are all reversible even after 12 hr. (159). With hard exercise of the legs in man the hepatic blood flow was decreased to about one-third of its usual volume, with a corresponding increase in the difference between arterial oxygen and that of the hepatic vein (160). Hepatic arterial blood flow affects sinusoidal pressure, as clamping of the arterial circulation produces a decrease in hepatic blood flow and a slight decrease in portal pressure. There is gradual recovery of the blood flow with continued lowered hepatic resistance (161).

Injected preparations indicate that when the portal circulation to the liver is eliminated the circulation through the arterial system of the liver increases within a few hours. A large part of the arterial blood enters the sinusoids, and the only juncture of arterial and portal blood seems to be in the sinusoidal capillaries (162). An increase of 7 to 13 cm. of water pressure in the portal vein caused filling of the collateral vessels, while greater increases of pressure up to 53 cm. were followed by a decrease in portal pressure within 30 to 60 min. with increased filling of the collaterals (163). As many as 15 collateral vessels may be demonstrated within a week after occlusion of the portal vein (164), which may be accomplished in the dog by partial constriction of the portal vein with iodized cotton threads (165). In man the severity of hepatic involvement of the portal system may be correlated with variations in portal tension (166). Portal blood may be labeled with fluorescein or  $I^{131}$  by colonic injection to identify the source of bleeding occurring in the gastrointestinal tract (167). Determinations of portal pressure, wedged portal pressure, and occluded portal pressure in patients with cirrhosis indicate that there is a postsinusoidal outflow obstruction that leads to an increase of hepatic sinusoidal pressure, which may exceed the portal-vein pressure and produce a reversal of flow from the liver into the portal vein; oxygen-saturation studies failed to indicate the presence of arteriovenous fistulas (168).

#### PRESSURE IN THE HEPATIC VEIN

With perfused rat liver, an increase of pressure on the hepatic vein exceeding the hilar pressure by 1 cm. or more of water rapidly distends the hepatic vascular tree and increases the volume of the liver by about 25 per



cent where it is limited by the distensibility of the capsule of the liver. The portal blood flow is reduced unless the portal pressure is increased. In addition to the distention of the hepatic venous system with blood, there are a sequestration of erythrocytes and an increase in extravascular volume which is found to represent extracellular fluid. Droplets of transudate appear on the surface of the liver. Analysis of the transudate, which is free of erythrocytes unless the pressure is made to exceed plus 4 cm. of water, shows that this fluid is similar to plasma. Bile flow is decreased by increased venous pressure, but the secretory pressure is not altered. The extraction of sulfobromophthalein or colloidal chromic phosphate is not altered by increased venous pressure except when the blood flow is reduced (169).

Increased hepatovenous pressure developed in dogs after gradual complete blocking of the hepatic veins by an occluding cannula in the vena cava (170), with ascites and portal hypertension developing as with other methods. Even without sodium intake, ascites develops in many dogs after constriction of the thoracic inferior vena cava. Plasma volume is not increased, and the excretion of aldosterone is increased and that of sodium reduced. The increased venous pressure initiates ascites formation, and the renal retention of sodium is a secondary phenomenon (171). The ascitic fluid weeps from the surface of the liver (172). After removal of the ascitic fluid in both dogs and patients with cirrhosis and ascites, the decrease in the measured volume of extracellular fluid became equal to the volume of ascitic fluid removed (173). Production of adhesions of the abdominal wall, omentum, and renal capsule to the liver by suture and aluminum silicate prevented the formation of ascites or reduced the ascites if already present (174, 175). Fibrosis of the capsule of the liver alone may cause the ascites to disappear (176). The application of a body cast to increase abdominal pressure causes a decline in the increased excretion of aldosterone and of sodium. This is considered a result of the lowering of the differential pressure between the liver and veins and may be analogous to the effects of hepatic capsular fibrosis (177). Various surgical means of draining the experimentally produced ascitic fluid have been tried (178), but eversion of a loop of ileum within the peritoneal cavity and removal of the omentum have been the most successful (179 to 181). Hypophysectomy will relieve the ascites caused by constriction of the vena cava, and no recurrence of ascites follows administration of ACTH, cortisone, deoxycorticosterone acetate, or Pitressin, but excess of salt will cause recurrence (182, 183).

Ascites does occur from constriction of the vena cava in dogs with experimental diabetes insipidus (184). Thyroidectomy in dogs with experimental ascites is followed by loss of ascites and increased excretion of sodium and water (185). Ligation of the left hepatic veins in dogs was about 50 per cent effective in producing ascites, and portal-caval shunt and other methods of reducing the blood flow to the liver were effective in reducing the incidence of ascites formation (186). The ascites developing in rats after vena caval constriction also is a transudate from the liver (187). Other mecha-

nisms of the formation of ascites are suggested because ascites may be produced in mice by the intraperitoneal injection of a killed suspension of *Staphylococcus aureus* mixed with Freund's adjuvant (188). With a pneumatic cuff around the thoracic inferior vena cava, immediate and reversible constriction has been produced. With inflation, there was rapid formation of ascites with prompt decrease in the urinary excretion of sodium and immediate reversal even after release of the inflation after 10 days (189). With a graft to shunt the portal-vein stump from the liver to the thoracic inferior vena cava in animals with an Eck fistula, reversal of flow could be demonstrated after occlusion of the vena cava below the graft. The animals survived up to five months; in some, small amounts of ascitic fluid developed before death with symptoms from the Eck fistula occurred (190). Vena caval congestion of the rat liver increases the retention of sodium after administration of deoxycorticosterone, and there is impaired ability of the congested liver to inactivate aldosterone (191). In cirrhotic rats, diuresis was inhibited by Pitressin to a much greater extent than in normal rats (192). In dogs with vena caval constriction the failure of mercurial natriuresis is not related to the retention of adrenocortical salt-retaining hormone (193). Patients with cirrhosis and ascites have an impaired ability to excrete aldosterone in the urine (194), and there is no evidence of change in the secretion of pituitary hormones (195). Infusion of sodium sulfate increases the excretion of sodium and potassium, decreases excretion of chloride, and does not change excretion of aldosterone in sodium-retaining cirrhotics (196), and expansion of the volume of extracellular fluid will also produce diuresis (197). The occurrence of antidiuresis from sodium chloride given during water diuresis when the serum osmolarity is low indicates release of antidiuretic hormone in a normal fashion, but at a lower level of osmolarity in patients with decompensated cirrhosis (198).

#### DIETARY HEPATIC NECROSIS

Diets that contain *Torula* yeast as the sole source of protein produce hepatic necrosis in several species of animals and a hemorrhagic exudative diathesis in birds. Species differences occur in mammals, but in all, the necrosis of the liver is a part of the systemic necrotic degeneration that involves many other tissues. Changes in the structure and arrangement of the microsomes of the hepatic cells of rats may be noted as early as seven days after the diet has been started. In the third week, slices of liver from deficient rats show a marked decline in oxygen consumption. There is also depression of the rate of removal of excess glucose from the blood, and the terminal phase is associated with hypoglycemia, at which time large necrotic lesions are present in the liver (199). The addition of aminoxidase inhibitors hastens the time of hepatic necrosis in rats (200), and a marked drop in the  $B_{12}$  content of the liver has been found as early as 15 days after start of the diet (201). Thyroid hormones also accelerate the rate of hepatic necrosis, but this

effect can be balanced by the administration of cortisone (202) or selenium (203) or aldosterone (204). The various regimens for producing necrotic liver have the common factors of being low in cystine and without vitamin E or factor 3, and the necrosis has been prevented by the inclusion of either of these in the diet. Selenium has been found to be an integral part of factor 3 (205) and to be present in L-cystine impurities in sufficient amounts to account for the protection afforded by the amounts of cystine required for protection (206). Other trace metals [cobalt, osmium, and molybdenum (207), and lead, cerium, mercury, titanium, and vanadium (208)] have been found ineffective. However,  $\text{Cr}^{+++}$  has been found in the factor necessary to maintain glucose tolerance, and several organic compounds containing  $\text{Cr}^{+++}$  are effective in this regard (209); also, this factor has been shown to be unrelated to the necrotic liver factor (210). The terminal hypoglycemia developing in rats with necrosis of the liver is related to the loss of hepatic function and not to the glucose-tolerance factor (211). Several antioxidants are protective against hepatic necrosis (212) and are protective in the same order against the respiratory decline (213, 214). A purified preparation of factor 3 prevents the exudative diathesis of chicks, but does not prevent fetal resorption or muscular dystrophy, each of which is easily prevented by vitamin E (215).

#### CIRRHOSIS FROM CHOLINE DEFICIENCY

The development of cirrhosis in rats fed a choline-deficient diet takes place in four progressive stages: the centrolobular cells contain fat, the entire lobule is fatty, newly formed cells are equally distributed in each acinar unit, and regenerative nodules are irregularly distributed (216). Regeneration, as indicated by a high mitotic index, occurs rapidly and continuously with the RNA content of the livers remaining constant and the DNA increased until the fourth period. The  $\text{P}^{32}$  turnover of DNA is increased, but declines in the fourth period also (217). With thymidine labeling, it can also be shown that formation of new cells precedes the cirrhotic changes (218). Fibrosis appears to develop as the result of necrosis (219) rather than from fatty cysts (220). Liver extract is 15 times as effective in prevention of connective-tissue formation as the amount of choline in the extract would be (221). Intact casein was more effective in preventing cirrhosis than the equivalent amino acid mixture, and a small amount of methionine prevented the accumulation of liver fat but did not alter the cirrhosis, which suggests that the two processes are not directly related (222). A choline-deficient diet produced cirrhosis in Cebus monkeys fed the diet with few intervals in 17 months, but an adequate diet for 9 months did not produce any appreciable improvement of the histologic picture of the liver (223). A number of hepatic enzymes were shown by histochemical means to be markedly altered in cirrhosis produced by ethionine (224). Increases in liver fat of female rats receiving ethionine are not accompanied by decreased levels of choline containing phospholipids, nor is the rate of synthesis of phospholipid altered.

Homogenates of ethionine fatty livers showed decreased ability to oxidize fatty acids, acetate, or pyruvate which was not produced by ethionine *in vitro* (225). Proliferation of bile ducts appears to be independent of change in the liver cells (226).

An experimental biliary cirrhosis has been produced by continued administration of alpha-naphthyl-isothiocyanate. This substance produces a diffuse hyperplasia of the bile ducts with epithelial buds which grow into the adjacent lobules of the liver and join others to make a network of ductules, blood vessels, and connective tissue constricting the lobules in a manner very similar to that in biliary cirrhosis. Liver function is not greatly disturbed and neoplasia does not occur (227). This lesion differs some from those produced by thioacetamid (228, 229), wherein it has been claimed that the primary lesion is the loss of selective permeability of the hepatic cell with subsequent influx of calcium which inhibits several enzyme systems (230). Allyl alcohol appears to produce a similar type of cirrhosis (231).

#### CARBON TETRACHLORIDE INJURY

Chickens tolerate large amounts of carbon tetrachloride injected subcutaneously, a characteristic that may be associated with their high body temperature. Extensive fatty changes occur in the liver of hens, but no change occurs in roosters (232). Fluorescein conjugated homologous whole serum or serum albumin given to rabbits or rats after damage to the liver has been produced is abnormally distributed in the injured cells of the liver with the fluorescence conspicuous in the viable-appearing cells bordering on areas of necrosis. The fluorescent droplets correspond to the acidophilic vacuoles and masses found in the balloon cells of carbon tetrachloride poisoning. Hydropic degeneration is caused in part by changes in cell permeability which allow plasma leakage into the cell (233). Active biliary secretion increases the necrotic effect of carbon tetrachloride in rats receiving taurocholate or glycocholate to increase biliary secretion (234). The total collagen in the liver of rats receiving carbon tetrachloride is increased threefold; the soluble collagen is related to the regenerative processes and the insoluble collagen to the fibrosis (235). Both cortisone (236) and ACTH inhibit the formation of connective tissue after administration of carbon tetrachloride (237), although there are no demonstrable changes in the adrenal gland until the late stages are reached (238). Carbon tetrachloride causes a marked decrease in the vitamin B<sub>12</sub> content of liver and there is an increased uptake of B<sub>12</sub> in the liver during regeneration (239, 240). Administration of B<sub>12</sub> protects against mitochondrial degeneration by carbon tetrachloride, but is not specific in this action (241).

After a single dose of carbon tetrachloride, studies of regeneration of the liver of rats by histologic methods and autoradiography of the nuclear uptake of thymidine indicate that new cell formation takes place in the nonnecrotic areas of the liver. There is no indication of regeneration until after about 24 hr., when a marked increase in the thymidine uptake is noted which is

followed in 12 hr. by an increase in mitotic figures. Both the mitotic index and thymidine uptake increase so that at 36 to 72 hr. the number of labeled cell nuclei is 250 times the control values that are again found at 120 hr. The diffuseness of the process suggests that it may arise from other than local necrotic factors, but plasma from rats at the period of greatest cellular proliferation failed to stimulate this process in other rats (242). The serum from partially hepatectomized rats given to rats receiving carbon tetrachloride appeared to reduce the amount of degeneration and increase the rate of regeneration (243). Some increase in the rate of regeneration after carbon tetrachloride has also been reported after partial hepatectomy in the same animal (244 to 246).

#### REGENERATION OF LIVER

The liver of lactating rats increases in size, and the DNA content of the liver is consistent with increased numbers of cells (247). Experimental nephrosis with albuminuria also causes the liver to increase in size (248). Functional loading of the liver (the addition of bile to the diet) increases the rate of hepatic regeneration after partial hepatectomy (249). Partial hepatectomy in 10-day pregnant rats gives an increase of about 20 per cent in early regeneration of the liver (250). After damage of the liver in birds from feeding the alkaloids of *Senecio jacobaea*, the females recovered rapidly, but little recovery occurred in males; however, estrogen injections aided the males to recover as fast as females (251). Regeneration of the liver of rats after partial hepatectomy was not altered in different age groups (252). The regenerative capacity of the liver is well maintained even after part of the liver has been removed every month for one year (253). Hypermetabolism does not alter the rate of hepatic regeneration (254). Thioacetamide stimulates synthesis of DNA, but does not interfere with regeneration except that the expected increase in the number of polyploid nuclei does not occur (255), and regeneration is good in lysine-deficient rats (256). Regeneration is more rapid in splenectomized than in normal animals (257). After 35 per cent hepatectomy, regeneration is more rapid in burned animals than in normal, but is equally rapid if 70 per cent of the liver is removed (258). Regeneration is slightly inhibited by intestinal antibiotics (259) and after administration of ethionine (260). Livers perfused 48 hours after partial hepatectomy secrete more bile in proportion to their weight and take up about twice as much colloidal chromic phosphate from the blood as normal livers (261). The tolerance to carbon tetrachloride is slightly increased (262), protein synthesis is not greatly changed (263), the formation of several antibodies is not altered (264), nor is the ability to take up and retain tobacco mosaic virus altered (265), during regeneration.

With the increasing weight of the liver after partial hepatectomy, there is an increase in content of total nitrogen, cytochromoxidase, acid phosphatase, and glucose-6-phosphatase. After 12 hr. the DNA content increases and after 24 hr. the number of nuclei increases. Compared with the number of nuclei

present, the increase of the above-mentioned substances is from 150 to 200 per cent, and a return to normal occurs after 48 hr. (266). There is an increase in the rate of uptake of precursors of nucleic acid by slices of the regenerating liver of hamsters; this differs in specificity from that found in regenerating liver from tumor-bearing hamsters (267). In regenerating liver, growth hormone accelerates the rate of DNA synthesis, and mitosis begins earlier (268); both DNA synthesis and mitosis are inhibited by x-radiation (269) and by amethopterin (270). A substance may be extracted from normal liver which inhibits thymine synthesis by bone marrow (271). Regeneration of the liver has been demonstrated by splenoportography (272, 273) and by microangiography which shows the increased blood flow through the regenerating liver (274). The lack of portal blood flow through the liver of monkeys after portal-caval shunt inhibited regeneration of the liver following partial hepatectomy which gave good regeneration in control monkeys (275). Normal serum did not inhibit regeneration in partially hepatectomized rats (276). Homogenates of liver or the extractable phospholipids of normal liver showed some slight inhibition of liver regeneration in rats (277). The regeneration of the liver after partial hepatectomy in one parabiotic rat was less than the regeneration in single controls (278). The mitotic index of regenerating liver was increased by serum from partially hepatectomized rats (279), and similar serum also increased the weight, the mitotic index, and the DNA of the liver when compared to control partially hepatectomized rats with or without injections of normal serum (280).

Hepatic physiology has been studied in a good preparation of the isolated perfused calf liver (281). The perfused rat liver has been used to identify the metabolic products originating from ethyl alcohol (282), and from amino acid and protein metabolism (283). Glucagon decreased the liver glycogen and increased the blood sugar (284), and appears to increase the entry of metabolic products from fat into the metabolic pathway between pyruvic acid and ketoglutaric acid (285). The relation of blood glucose to the liver has been recently reviewed (286).

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## SKIN<sup>1,2,3</sup>

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This is the fourth review of the Physiology of the Skin to appear in the *Annual Review of Physiology* since 1946 (1, 2, 3); the most recent was published in the 1958 volume. Since then the areas of research that encompass the skin have been increasing rapidly. As a result, so much new information is available that it is impossible to do justice to it in a review such as this. Fortunately, reviews, monographs, and the published results of conferences, unifying the advances of the ultrastructure and function of the various anatomical elements of the skin, either have been published recently or are in the process of preparation or in press at the time of this writing. We prefer first to refer you to these sources of current critical information. They are as follows: hair (4, 5), human integument (6), apocrine glands (7), pain and itch (8), biological sciences in relation to dermatology (9), pigmentation (10, 11), biochemistry (12), eccrine sweat glands (13), pharmacology (14), metabolic processes (15), and metabolism of oral tissues (16).

As such, then, this review will not consider keratinization, sebum and surface lipid, or connective tissue (15); nor vascular physiology and the physiologic and pharmacologic mediators of inflammation (6, 9, 13, 14). Sensation will be discussed only from a limited aspect (6, 8, 9).

The skin, as the organism's side of the interface between the organism and its environment, has several functions of physiological, behavioral, and ecological importance. In this review we would like to stress some of these aspects of cutaneous function. For such functions the skin: (a) acts as a selective barrier to some substances and transmits others, (b) acts as a selective barrier to some forms of energy and transmits others, (c) acts as a barrier to some types of information and transduces others.

### A BARRIER AND TRANSMITTER OF SUBSTANCES

The skin has been studied as an avenue for absorption as well as a barrier to penetration of water, electrolytes, anionic substances, and mediators which affect or block physiologic responses. These studies have been carried out *in vivo* and *in vitro* with animal and human skin, with skin that is intact or purposefully altered, and with skin from various body areas.

Ideally, the techniques employed should permit determination of (a) the number of molecules which penetrate a unit area of skin in a unit time, (b) the ultimate destination of the molecules, and (c) their good or bad effect on the skin locally or on the various systems generally (17).

<sup>1</sup> The survey of literature pertaining to this review was concluded in March 1960.

<sup>2</sup> Among the abbreviations used in this review is MSH (melanocyte-stimulating hormone).

<sup>3</sup> This work was supported in part by U. S. Public Health Service, N.I.H. grants C-5052 GM(1) and E-3102.

The rate at which the molecules move within a vehicle (diffusivity) or in any phase of the skin will influence the rate of penetration. The chemical reactivity of a substance also influences its ability to penetrate the skin. If an agent reacts easily with certain skin constituents, it will be held by those constituents and its movement will stop. Also, the structure of the skin may be altered by chemical reaction or by the removal of various constituents by solvent action. Either of these alterations in the skin may be produced by the penetrant or by the vehicle in which the penetrant is held. "Intactness" of the skin is one of the most important factors which affects penetration.

Blank (17) points out that most of the methods used for studying percutaneous absorption at present fit into the following classification: (a) use of "models" into which penetration takes place, (b) study of changes of histology of the skin, (c) use of tracers (dyes, fluorescent compounds, radioactive elements), (d) measurement of physiological reactions, (e) analysis of tissues: skin, blood, urine, etc., (f) measurement of loss of penetrant from the surface, and (g) measurement of penetration into perfused skin.

The degree of molecular interaction between a penetrating substance and a membrane determines the nature of the permeation mechanism. Unfortunately, the skin as a membrane to be penetrated is not yet so simply defined. Avenues for penetration of the epidermis may be three: (a) through the pilosebaceous structure, (b) through the sweat ducts, and (c) directly through the epidermis.

The evidence is increasing that the physical barrier in the skin to penetration in either direction is a layer, a few microns thick, at the base of the stratum corneum. Szakall (18, 19) was able to remove this entire barrier with a single stripping, using Tesa tape, and to recover this membrane by dissolving the tape in petroleum ether. Mali (20) isolated the "barrier layer" intact by scraping the stained stratum corneum and rete layer after maceration of the skin for two days in a 1 per cent methylene blue solution. There is electronmicroscopic evidence that the ultrastructure of this area, lying between the stratum granulosum and stratum corneum of the epidermis, is made up of fine, closely packed, fibrillar structures contained within cell outlines (21 to 24). Two separate processes seem to occur in and just external to the stratum granulosum; one is probably keratinization and the other results in nonfibrillar material and, strictly, is not a keratinization (22).

Very intense staining for sulfhydryl compounds can be demonstrated in this barrier area, and they differ from other sulfhydryl groups in that they (a) bind maleimide in such a way as to leave it free to combine with H acid (*L*-amino-8-naphthol-3,6-disulfonic acid), (b) stain with dinitrofluorobenzene much more intensely than with Bennet's reagent or by the Barnett-Seligman method, and (c) are not differentiated by tetrazolium (25, 26). Stoughton (26) adds that the relation, if any, of the high thiol content of this area to its barrier-like properties is not clear. This area also is stained with the Gram stain. In normal epidermis with a thin horny layer, the Gram-stained portion is restricted to a narrow band between the Malpighian and



horny layers. In regions with highly developed stratum corneum, not only the lowermost zone stains, but there is scattered Gram staining as well, especially around the sweat ducts (27). Chemical studies show that this Gram staining could not be abolished by any solvent, "keratolytic" and oxidizing agents, acids, enzymes, and sulfhydryl inhibitors that were used. The only substances which consistently blocked the staining were mercuric acetate and ferric salts. There is a parallelism between the degree of Gram staining and the glucosamine concentration. Thus, this material coinciding in location with "the barrier" may be a complex, highly resistant acid mucopolysaccharide. The following enzyme activity has also been localized histochemically in and around this so-called "barrier" area of the epidermis: nonspecific esterase (but not in palm skin) (28 to 30), acid phosphatase (31), phosphamidases (32), phospholipids (33), and  $\beta$ -glucuronidase (34). The purpose of such activity may be only in manufacturing the epidermis from the stratum granulosum outward. It is possible that such enzyme systems may play an active role in effecting a "barrier" or in aiding a transepidermal transport.

Electrical conductivity measurements (to one volt of direct current) can detect small breaks in this major barrier in the skin (35). This can be done by a simple method of holding the excised skin directly between two 16-mm. electrodes. Only in skin of low conductivity (1  $\mu$ amp.) is the barrier intact. This conductivity was correlated (36) with the effect of serial stripping with adhesive tape wherein successive layers of the stratum corneum were removed. Between six and twelve strippings caused the conductivity to rise from the control of 1  $\mu$ amp. to 17  $\mu$ amp. Twenty-four strippings raised this to 65  $\mu$ amp. The rate of repair of the barrier, too, can be followed by periodic measurements of the conductivity of injured sites. Superficial scratches heal within 24 to 48 hours (36). Thus, part of the skin's protective function depends on rapid reconstruction of "the barrier".

Many studies on repair of the epidermis to "strip" injury have been carried out since this technic was first described by Wolf (37). This technic was facilitated by a small machine for rapidly and conveniently carrying out the essentially painless stripping procedure. The entire stratum corneum including the water and electrolyte barrier layer is removed when uniform glistening appears on the surface of the skin. No additional layers and no living cells are removed if stripping continues beyond this point. Lorincz's studies (38) on man compared *in vivo* responses of the intact skin with the skin that was stripped to "glistening". Topical applications of droplets of 1:1000 histamine phosphate, 1:1000 epinephrine hydrochloride and surgical Pitressin were applied to these stripped areas. The classical blanching that occurred immediately at the epinephrine and Pitressin-treated sites and the usual itch, wheal, and erythematous flare that occurred at the histamine sites indicated free penetration of these agents to the blood vessels of the dermis. Similar applications of these same agents to intact skin failed to produce any of these responses. Further investigative studies involved the urticario-

genic effect of trypsin and histamine phosphate; in comparison to the histaminic wheal, the wheal lacked the axon-reflex flare, developed no sensation, evolved more slowly and persisted longer, and was not inhibited by previous topical application of diphenhydramine. Such responses to topically applied pharmacophysiologic agents can also be used to study the rate of re-formation of the water-electrolyte barrier. Using 1:10,000 histamine phosphate periodically as the test agent, approximately 24 hr. were required for reformation of an effective barrier to this salt. The same technic can be used to study the specific allergic reactions of the skin. The contact eczematous response of a subject with known nickel sensitivity was more severe on a stripped site than on the intact skin; but pentadecylcatechol in acetone produced only a very minimal diffuse scaling and erythema in the stripped site, whereas the usual severe reaction occurred within 72 hr. on intact skin in individuals sensitive to poison ivy. As one would expect, immediate wheal-producing antigens did so readily on stripped skin and not at all on intact skin. An individual with severe solar urticaria (3300 to 4500 Å spectrum) failed to react with whealing when the stratum corneum was first removed by stripping. The intense whealing did occur on exposure of the intact skin. Possibly a photochemical antigen or metabolite is produced in the layers removed.

Monash (39) controlled his work on human skin by taking repeated biopsies and found that removing one-half to two-thirds of the outer stratum corneum (10 to 15 strips) of the volar surface of the forearm removed the main barrier to penetration of 2 per cent procaine HCl and 2 per cent xylocaine HCl and histamine base solutions. The remaining stratum corneum, including "the barrier" isolated by Szakall (18, 19) and Mali (20), offers very little resistance to the passage of anesthetic and histamine solutions. The barrier to these solutions on the palms and soles differs, however. Here 98 per cent or more of the stratum corneum (600+ strips with Scotch tape) must be removed; thus the barrier is much thicker and consists of practically the entire thickness of the stratum corneum. The time involved for the penetration of a local anesthetic also can be used to study the time necessary for the re-formation of the barrier, and by combining these experimental devices *in vivo*, the location and re-formation of the epithelial barrier to insensible water loss (water vapor) can be sought. The barrier to insensible water loss is mainly in the inner two-thirds of the stratum corneum (40). The epidermis below the level of the stratum corneum is a wet tissue from which water will evaporate at approximately the same rate as from a free water surface. The re-formation of the stratum corneum barrier to increased insensible water loss is very rapid, requiring only 48 to 72 hr. The re-formation of the stratum corneum barrier to superficial epithelial penetration by an anesthetic solution is a gradual one requiring about two weeks, or until the formation of a normal stratum corneum is complete. In this problem of barrier repair, it is interesting that a second removal of the healing stratum corneum by the strip technic at intervals varying from 4 to 48 hr. after the first removal did not alter the fundamental response of repair for the living

epidermis. Not until 72 hr. had elapsed after the first strip could the cytological cycle of repair begin again (41, 42).

About 2 per cent of  $I^{131}$  applied to the human forearm "passed into skin barrier" and was detected in the thyroid and urine (43). Penetration of  $I^{131}$  through palmar skin (devoid of hair follicles) was also demonstrated, being about one-third of the penetration through the skin of the forearm. About 7 per cent of the amount applied to the forearm and 13 per cent of the amount applied to the palm remained on the superficial layer of the skin for a period of at least 48 hr. Kutzim (44) found about 10.7 per cent absorption of  $NaI^{131}$  in normal skin after 24 hr. Whether iodine penetrates the skin as the iodide ion or as molecular iodine is uncertain. With a pH higher than seven, no conversion to molecular iodine will occur. The percutaneous absorption of cortisone-4- $C^{14}$  acetate has been established by detection of radioactivity in the urine; according to these studies cortisone and hydrocortisone penetration are similar qualitatively and quantitatively (45).

Penetration studies have used the anticholinesterase compound isopropylmethylphosphonofluoridate (Sarin) because of its biological effect after absorption, because it can be followed with autoradiographic studies, and because one can determine by direct chemical measurement the amount of substance which has penetrated (35, 46 to 48). Penetration occurs across the epidermis, not preferentially through hair follicles. In this instance, the total stratum corneum is the barrier. Intactness of the barrier is important in limiting the penetration of Sarin through the skin. Sarin does not penetrate nearly as rapidly as water ( $350 \text{ m}\mu \text{ M per cm.}^2 \text{ hr.}$  for Sarin versus over  $5000\text{--}20,000 \text{ m}\mu \text{ M per cm.}^2 \text{ hr.}$  for water) and seems to penetrate the "barrier" without assistance of any energy derived from living cells. But the time of absorption per unit area is limited and not affected by a mere increase in the amount applied. Such discrepancies cannot be blamed on the barrier, however, because these organophosphorus compounds are liable to hydrolysis and it has been shown that there are enzymes present in the skin capable of hydrolyzing them (48).

The penetration of anionic surfactants (sodium laurate and sodium dodecyl sulfate) through excised human skin has been studied. Analysis of the radioactivity of the stripped-off layers of stratum corneum after the 18 to 24 hr. application of radioactive surfactants (temp.  $20\text{--}25^\circ\text{C.}$ ) showed that a major portion of them is in the first two strips. Perhaps the proteins of the stratum corneum bind these compounds as at least one mechanism in preventing penetration (49).

Can an oral or systemically given chemical be recovered in various layers of the stratum corneum (i.e., penetration or diffusion from within out)? Again, with the use of the "strip" technic to selectively remove 25 per cent, 50 per cent, 75 per cent, and 100 per cent (to "glistening") of the stratum corneum of the human flexor forearm, the "transport" of the orally administered antibiotic griseofulvin could be followed because of its biological effect on germination of the topically applied macroconidia of *Microsporum gyp-*

*seum*. The average time for the appearance of the griseofulvin effect was 4 hr. in the dermal blood stream, 62 hr. at the "glistening" level, 9.5 days at the 75 per cent removal level, 15.6 days at the 50 per cent removal level, and 33.2 days at the intact surface of the stratum corneum (50). These data correlate exactly with those on the normal growth rate of the epidermis. Thus, it would seem that this chemical does not diffuse across epidermal cells but once it has entered the basal cells of the epidermis is transported along with them to the surface of the skin.

Water can pass into the body through skin by exchange, but there is no net transfer inward. The inward passage has been demonstrated by experiments with deuterium oxide and tritium oxide. Water penetrated into the skin of living man at the rate of  $1.5 \text{ m}\mu \text{ M per cm.}^2 \text{ hr.}$  Tritium oxide diffusion rate out of the skin equals the tritium oxide diffusion rate into the skin (36, 51 to 53).

The nonsweating water loss through the skin (water of diffusion, insensible water loss, insensible perspiration) is still subject to continued (and inconsistent) investigation. Griesemer (36) summarizes some of the studies done on living man and excised human skin from comparable areas and at comparable temperatures. The variations were such that no consistent conclusions could be developed. He also states that there is entirely too much variation in the penetration values of the small group of substances already studied to permit general conclusions for all classes of substances. By isolation of the barrier zone, Mali (20) demonstrated that it is in truth this zone (the stratum corneum junction of Szakall) which determines the small inward and outward permeability for water vapor. With it he showed that transepidermal water transport is directly proportional to the vapor pressure gradient across the membrane and inversely proportional to its thickness. At varying environmental temperatures it can be shown that the permeability increases with temperature. Water transport through the plantar skin was six times greater than through the skin of the trunk [ $0.5 \text{ mgm. per cm.}^2 \text{ hr.}$  (trunk) versus  $3.0 \text{ mgm. per cm.}^2 \text{ hr.}$  (plantar)]. There is also evidence, not yet confirmed, that permeation of water vapor through the isolated membrane from the sole can be accounted for entirely on the basis of diffusion of water vapor through sweat ducts. In comparison, the transepidermal water loss constitutes only about 20 per cent of the total insensible water loss from living skin (20).

Of course there is a water-holding component in the stratum corneum (significant in scale formation, desquamation, etc.) which may play a role in water diffusion. It makes up about one-fifth of the horny layer, is a globular protein, and may be abnormal in the presence of normal keratin protein formation (54). Extremely significant observations (55 to 57) along these lines were made pertinent to both sweat gland function and water loss. Hydration of the stratum corneum tends to inhibit sweating on the palm and sole, the amount of inhibition being related directly to the amount of hydration. These *in vivo* studies in man also showed that the uptake of water by the

skin when immersed in various solutions is primarily a process of hydration of the stratum corneum. The amount of water uptake on the palm and sole exceeds that on other skin areas and appears to be related to the amount of cornification of the skin. The rate of water evaporation from the skin surface is increased by the hydration of the stratum corneum in the presence or absence of sweating.

Some water diffusion studies have been done (*in vivo* and *in vitro*) by putting water vapor against the skin and others by putting water liquid against it. The resistance of skin to the passage of water was estimated to be about ten times as great for vapor contact as for liquid contact (58). Untreated stratum corneum of living skin absorbed both liquid water and water vapor; however, stratum corneum that had had water-alcohol-soluble and ether-soluble components extracted from it repelled the liquid water, but absorbed the water vapor. The extracted stratum corneum released the absorbed water vapors almost three times faster than did the untreated stratum corneum. It seems, then, that the water and polar-solvent soluble components of keratin structures are responsible for the liquid water uptake and the water-and-moisture holding capacity of the "horny layer" and that the sebum regulates the evaporation rate and protects the water-soluble compounds from being washed out (59).

Even barometric pressure might have some influence on the loss of water (weight) by diffusion. In a carefully controlled environment (but the observations for sweat gland activity could not be considered adequate) the total body weight loss in a "sweat-free subject" is inversely related to both water vapor pressure and barometric pressure. The calculated insensible weight loss of skin thus is also inversely related to barometric pressure but is not clearly related to vapor pressure (60).

Buettner ("Our statements differ from the generally accepted aspect of skin diffusion or rather lack of it") (61 to 64) discusses many of the problems of water loss from the skin as he summarizes his present work.

Water and water vapor are believed to move through intact skin surface by: (a) glandular secretion such as sweat gland, (b) sorption and desorption in the horny layer, (c) diffusion through the horny layer. With proper care all three avenues can be sufficiently well differentiated. Sweating can be partly controlled by low room temperature, atropine and selection of a body area not prone to sweating. Sweat amount is also measured in blank tests on the opposite limb, furthermore by counting sweat droplets. Horny layer sorption and desorption is a saturation process after the skin enters a new environment. After sweat and sorption are evaluated or prevented, there remains a flow of liquid water or vapor through the skin. This flow seems to depend on the water concentration of the medium touching the skin. This concentration will be defined for water solutions, as well as for air, as relative humidity  $r_a$ . In all but a very few test persons water and water vapor, from solutions or air, of more than  $r_a=90$  per cent, pass into the skin. Since the point of no transfer of 90 per cent relative humidity or about six osmolar exceeds by far the body osmolarity, the transfer should be active.

The active process or pump seems to be separated from the environment by a

barrier. This barrier appears to be part or the whole of the stratum corneum conjunctum. Barrier and pump seem to be different entities with the following characteristics: (A) the barrier: its resistance is about ten times higher on arm or leg than on palm or sole; about 3-5 times higher for the same skin area under dry than under moist conditions; invariant to four hours of ethyl ether exposure; absent for about three days after stripping off the stratum conjunctum. (B) the pump: its intensity is increased, (i.e., the neutral relative humidity is lowered) in persons having edema from toxemia, pregnancy, internal disorders and menstruation; the pump intensity is lowered, (i.e., the neutral relative humidity is raised to nearly 100 per cent) in myxedema; after skin stripping; in two of our persons after a four-hour exposure to ethyl ether. After stripping the pump comes back to normal in about six days. The rubefacient nicotinic acid did alter neither barrier resistance to water vapor nor the pump [(64)].

For the present one wonders, then, in light of all this new information, whether one should state "that the rate of water loss is a simple function of the vapour pressure gradient from saturation at approximately skin temperature to ambient water vapour pressure" (65).

#### ENERGY BARRIER AND TRANSMITTER

Under this heading we will discuss heat transfer, exposure to hot and cold environments, and the effects of visible and ultraviolet light. Ionizing radiation, mechanical forces, and ultrasonic vibration are no less important but are not considered here.

The problem of heat transfer into the skin by conduction and by radiation is admitted to be complicated by all who have investigated it. The tendency toward a maximum of computation and a maximum of assumptions by the biophysically oriented physiologists is viewed with skepticism by the more anatomically and biologically oriented investigators. That these viewpoints will be resolved into a continuum of viewpoint and knowledge is indicated by the eloquent pleas for more knowledge of the optical, thermal, and functional characteristics of the different skin layers, which come from the biophysical investigators (66). In this report Stoll remarks of the skin,

Less often it is thought of in terms of its own thermal function, although it is in itself a heat generator, absorber, transmitter, radiator, conductor, vaporizer and detector. . . . Most of the data available date back to the 1800's and are of questionable accuracy, largely due to the technical difficulties of assessing the dimensions of irregular heterogeneous tissue. . . . It develops then that the role of skin in heat transfer is manifold. It may be considered to be: a multiple-layer covering of a heat source through which heat passes at a measurable rate; a servo-mechanism for the regulation of this heat passage; an alarm system to detect and transmit signals indicating dangerous extremes of temperature; a living organ generating, dissipating and modulating the flow of heat between the entire organism and its environment; and finally, an indispensable, vulnerable, yet highly regenerative and adaptable thermal integument having remarkable ability to alter its properties, thus inhibiting heat loss in the cold and augmenting heat loss in the heat.

Hardy, Stoll, and their colleagues have continued to study heat transfer in the human skin and the relationship between skin temperature and pain

(67). In their recent studies they have used apparatus which has permitted continuous recording of skin surface temperature throughout the period of exposure to a beam from a tungsten lamp. The skin surface is painted with carbon black to localize absorption of radiation to the surface. From the temperature rise of the surface, absorbed energy, and the thermal conductivity, density, and specific heat of skin, a number of relationships to pain threshold and to blistering injury were computed. The  $K\rho c$ , the product of thermal conductivity  $\times$  density  $\times$  specific heat, was found to be variable and to increase with increasing levels of irradiance. On the assumption that the pain receptors fire at some definite temperature, the authors calculated the depth of these receptors to be approximately 200 microns and to have a threshold of approximately 43.2°C. The authors compare this value to other work (68) in which a surface temperature of 43.7°C. was found for threshold pain on very slow heating. A value of 43.8°C. was found when the subject could adjust the irradiance to maintain the threshold pain sensation. The relationships between tissue temperature, units on the pain scale, and the time required for blister formation are also given (67).

Neisser (69) has also studied pain sensitivity of normal subjects with a device which measured surface temperature constantly during radiant heat application. He was particularly concerned with the variation and errors in such studies related to variations from point to point on the body surface. He found that the pricking pain thresholds were lower than those reported by Hardy, Wolff, and Goodell. He attributes the difference to his use of untrained subjects.

Because of the practical problem of flash burns, several groups have been studying heat transfer in the skin in relation to very-high-intensity thermal radiation sources. Derksen and colleagues (70) give a discussion of the considerations in this type of study which is of considerable help to the general reader who may be overwhelmed by the mathematical considerations inherent in heat flow computations for irradiated opaque and diathermous solids. They compared the temperature rises with the skin alone and painted with carbon black. Vendrick & Vos (71) compared stimulation of warmth sense by infrared and microwave radiation; they concluded that a threshold sensation of warmth is produced by a given rise in temperature of the temperature receptors. In another interesting study on the relationship between pain and tissue damage (72), it was demonstrated that the hyperalgesia following ultraviolet radiation produced a lowering of pain threshold to heating by as much as 6°C., but had only a slight effect upon the quantity of radiant energy required to produce blistering.

Belding (73) has studied heat transfer from heated pads through various areas of human skin. Skin conductance approached a maximum at 40°C., indicating a maximum blood flow rate. Of 12 skin regions studied, the face accepted most heat at 40°C. In view of the practical aspects of keeping people warm by localized input of heat, it is of interest that the pain threshold was reached with lower skin temperatures in areas with higher conductance, with



the result that maximum tolerable heat input was about the same for different regions. Average conductance for large pads indicated that sustained blood flow to the skin must be at least 1.4 liters per minute in hot environments.

In addition to studies on the biophysics of heat transfer, work has continued in many laboratories on the effects of environmental heat, particularly as it relates to sweating. Some of the most dramatic of these findings are covered in another review on the subject of sweat glands (13). In addition, the study of various reflex mechanisms leading to localized sweating or suppression of sweating has been continued, particularly by Japanese and Soviet investigators. Karaev (74) studied the effect of oral and gastric stimulation with hot and cold beverages, pepper, and quinine. He counted sweat glands on the palmar surface of the fingers, an area of psychic rather than thermal sweating. This is supported by Karaev's findings on response to hypnosis in which the ingestion of hot, spicy foods was suggested to the subject. Kaladze (75) studied the effect of heating the arm with the circulation arrested. In some subjects this caused arrest of sweat secretion in the opposite limb. He concluded that warming of the sweat center by warm blood is apparently more important than reflex sweating from locally heated areas.

Nakayama & Takagi (76) used a continuous recording method for localized sweating. They demonstrated that the periodic variations observed occurred concomitantly on some symmetrical parts. Unilateral skin pressure decreased the rate of sweating on the same side, and after a short latent period, sweating on the opposite side increased. Bernstein & Sonnenschein (77) have supplied other evidence of the cyclical nature of the central stimulus to sweating. They found that the local injection of tetraethylammonium ion produced cyclic local sweating and piloerection apparently because this ion potentiates the background tone of the nerves innervating the cutaneous effectors.

Bass and his co-workers (78, 79) have continued their studies on sweat composition as affected by local environmental factors. They have compared sweat composition as collected by the most common method, that of an impermeable bag, with sweat accumulated on the skin surface in a temperature-regulated box and reconstituted to the volume of water loss. The differences obtained indicate considerable error in the impermeable bag method. Contrasting effects of humidity and temperature upon sweat composition were reported.

In still another in this sequence of studies indicating the extreme sensitivity of sweating to local and central influences and the subsequent care required in experimentation and interpretation, Randall and colleagues (80) have reported further on their studies of recruitment of sweating in different skin areas as environmental temperature is raised. Active sweating began on the feet and progressed upward; the sensation of warmth began on the face and spread downward.

Robinson (81) studied the interrelationships between environmental temperature, exercise, and sweat rate. In a constant environment, rectal temperature and sweat rate varied directly with metabolic activity, whereas skin temperature tended to remain constant. Increasing mean body temperature and decreasing gradient between rectal and skin temperature were the best single indicators of the magnitude of the thermal stimulus for sweating.

Fox & Hilton (82) suggest an interesting relationship between sweating and heat vasodilatation. They interpret their findings as indicating that bradykinin release from the eccrine sweat gland is a major factor in active vasodilatation in human forearm skin. Wolff's group has also studied the release of local humors in vasodilatation (83, 84) and in the axon reflex flare produced by noxious stimuli. They describe release of protease that acts on globulins to form polypeptides which may produce itching, lower the pain threshold, and initiate the changes of inflammation.

Studies on the physiological effects of cold and acclimatization to cold environments have shown some shift of emphasis from the endocrine glands to local changes at the body surface. Renbourn (85, 86) in the United Kingdom and the Quartermaster Group (87) in the United States have searched for changes which would explain the alleged chilling effect of damp cold as compared to dry environments. It was concluded that under chamber conditions without a radiation temperature difference from air temperature, at temperatures of 40 to 60°F., no effect of humidity on subjective response, or skin or rectal temperature could be found in either nude or clothed men. In general, therefore, humidity appears important in heat loss from the skin only when evaporative cooling of sweat is required for temperature regulation and under colder conditions where insensible perspiration becomes a significant avenue of heat loss.

A long-standing practical problem has been the use of handwarmers for men exposed to cold environments. The claims of users seemed out of proportion to the very small quantity of heat which could be transferred to the body by this means (88). This discrepancy has apparently been resolved in favor of the handwarmer by the studies of Gaydos & Dusek (89, 90). It was found that the lowering of local hand and forearm temperatures was primarily responsible for the impairment of complex performance in the cold. Decrease in manual dexterity occurred with local cooling even though the body was warm; if the hand was warmed, dexterity remained normal even though the body was cooled. Manual proficiency dropped when hand skin temperature dropped below 50-55°F. and remained normal as long as hand skin temperatures were maintained at 80°F. or higher.

Leblanc has studied some of the skin changes in animals exposed to cold. Increases in mast cells were observed in abdominal skin and internally, but a decrease was seen in the exposed ear of rats (91). Thickening of the stratum corneum and the entire epidermis was shown in rats and goats exposed to cold environments (92).

The circulation to the skin is a major aspect in heat transfer, as well as in the physiology of the skin in general, but is omitted because of the review of peripheral circulation by Bohr in this volume (93).

High-intensity sources of non-ionizing radiation, usually combining infrared, visible, and ultraviolet light, have been used to study radiant heating of the skin as described in previous paragraphs. Smaller doses of visible and ultraviolet light in which the thermal effects are not overwhelming produce photochemical effects which are also of interest. There is a rapidly expanding literature on the subject of photobiology (94, 95), the related biochemical considerations of photoactivated molecules, and the role of free radicals in metabolism (96). The series of books edited by Hollaender (94, 95) is a good introduction to the more recent advances.

A review of the physiological effects of sunlight on animals and man has been published (97). Many general physiological effects of ultraviolet and visible light have been studied in other animals. What relation these may have to the highly touted beneficial effects of sunlight on human health will have to await appropriate investigation.

While most of the effects of light on animals are believed to be mediated through the eyes, some undoubtedly occur in the skin. The demonstration of the site of vitamin D synthesis in human skin is discussed under pigment.

Claesson and co-workers (98), by the use of photolysis flash lamps, have greatly extended the reciprocity law by which equal reactions to ultraviolet are produced by a constant product of intensity and time. The previous validity over a twenty-fold range was extended to a 10-million-fold range of intensity for ultraviolet at 310  $m\mu$ .

Further attempts to determine the nature of the vasodilator material released in the epidermis by ultraviolet light have continued to eliminate histamine but have not yet identified the materials producing local erythema (99, 100). However, some of the systemic effects of ultraviolet irradiation appear to be related to histamine.

Even if histamine has no direct role in sunburn erythema, its precursor, histidine, is probably involved with the skin's reaction to ultraviolet light. Urocanic acid that is formed from histidine by an alternative metabolic pathway has been found to be an important ultraviolet absorber in sweat (101, 102). It is also present in the skin and could be identified as being responsible for the considerable ultraviolet absorption at 265  $m\mu$  of the water-soluble extracts of the peripheral horny layer. The urocanic acid content of the peripheral horny layer was found to be up to 2 per cent (103). A second ultraviolet-light-absorbing substance was found that acts similarly to urocanic acid, the concentration of which is higher in the deeper than in the superficial horny layer. This substance is similar to but can be distinguished from urocanic acid by paper chromatography (103). Histidine is also believed to be present at the active site on the tyrosinase molecule (104).

Claesson & co-workers (105) have studied the action of ultraviolet light on skin with and without the horny layer. They reported that at both 2537 Å

and 2800 to 3300 Å the areas from which the stratum corneum had been stripped with adhesive tape showed a more marked erythema response. They could not confirm the report of Rottier & Mullink (106) that the reaction to 2537 Å radiation requires the presence of the stratum corneum, suggesting different mechanisms for the sunburn from short ultraviolet compared to middle ultraviolet.

Several investigators applied photoelectric measuring technics to improve the quantitative determination of ultraviolet effects on human skin over the traditional "minimal perceptible erythema" and provided quantitative descriptions of the operating characteristics of ultraviolet erythema with and without chemical photosensitization (107 to 109). For many studies, the photoelectric devices for evaluating erythema provide greater sensitivity and more information than temperature measurements.

The subject of photodynamic action (110) and photosensitization of human skin by drugs has been extensively studied. Photosensitization of human skin and other biological systems is discussed fully in a series of papers in a special issue of the *Journal of Investigative Dermatology* (111). A wide variety of compounds which can produce photosensitization is discussed. The furocoumarins (psoralens) are of interest both in animal pathology and plant physiology. These compounds photosensitize many biological systems at ultraviolet wavelengths around 3600 Å (112, 113). Applied or ingested, they photosensitize human skin (114), producing erythema, heightened pigmentation (115), and increased epidermal thickness (116). These compounds are found not only in several medicinal plants but also in many food plants such as celery, figs, parsnips, and limes. They have been described particularly from the *Umbelliferae*, *Moraceae*, and *Rutaceae*, with some species from other families. In plants they act as growth and germination regulators (117). They also produce vasomotor changes which are evident before the onset of erythema and vasomotor and sensory changes, some of which persist for months (118).

A considerable proportion of mammalian laboratory investigation is done on animals with a conspicuous genetic defect, namely, the absence of the enzyme tyrosinase in the melanocytes of the skin, hair bulbs, and eye structures. The total of the physiologic implications of the selection of albino animals for biochemical, nutritional, and physiologic research is not clear. The convenience of using animals which are visibly homozygous for a recessive gene is apparent. Certainly, the quantity and distribution of melanin pigment throughout a major portion of the animal kingdom has many ramifications in terms of camouflage, recognition, predation, and natural selection. Anthropologists have come to recognize that the differences in pigmentation seen in human populations may represent not genetic persistence from primordial stocks, but the effects of continuous selection pressures in which pigmentation or its lack may have genetic survival value in different environments (119). However, the specific means by which selective survival accrues may be very hard to determine (120). The industrial melanization of moths

in England has provided a clear-cut example of natural selection occurring within the hundred years since Darwin's *Origin of the Species* was written. The endocrine and other regulators of melanin quantity and distribution were the subject of notable contributions during the period of this review. Various hormones affect the skin and its pigmentation (10). Three of these, alpha and beta melanocyte-stimulating hormone (MSH) and melatonin, have had their chemical structures determined by Lerner and his co-investigators. The chemistry of the melanocyte-stimulating hormones has been reviewed by Lerner & Case (121) and by Harris (122). Under the earlier names of intermedin, melanophore-expanding hormones, and chromatophorotropic hormone, these hormones have long been of general biological interest. The sequence of the thirteen amino acids in alpha-MSH was found to be identical with a sequence occurring in hog and sheep (123) and in human corticotropin (124). MSH produces darkening of human skin and hence may have melanin-dispersing effects in human melanocytes as well as frog melanophores (121), although this question remains unsettled.

The action of MSH on frog melanophores has led Lerner to suggest that hormones in general may require intact functioning cells for their actions and that relevant hormone effects upon isolated enzyme systems need not be expected (125). In the case of melanin granules, the effect of the hormones and humors leading to dispersion of the granules involves conversion of the cytoplasm from a gel to a sol with the expenditure of energy. For aggregation the cytoplasm changes from a sol to a gel, and oxygen is not required for this reaction. Since the dispersion and darkening are followed by an increase in the total amount of melanin, Lerner suggests that similar control over the location and dispersion of metabolically active granules may be a general property of hormonal regulation, which is conspicuous in the case of melanin because of the blackness of the granules. It is argued that the dispersion and aggregation of the metabolically active granules will change the enzyme-substrate relationships. The associated gel-sol changes may also have an effect upon the enzyme-substrate accessibility. It is hardly news to physiologists that the physiology of intact cells, intact organs, and intact organisms cannot be explained entirely from the *in vitro* reactions which can be studied on fragments. It is of interest to see a theory which explains in part the effects of a hormone in a highly organized biological system. It is of further general interest to see the progress since the first quarter of the century, when pituitary effects on pigmentation were described by ablation and replacement studies (126), through the isolation of pure compounds, elucidation of their chemical structure, and, now, current interest in their specific actions upon the responding cells.

There are many compounds which disperse melanin granules in melanophores. Of these melanocyte-stimulating hormone is the most potent. Others include ACTH, caffeine, and progesterone. Aggregating, i.e., lightening agents, include norepinephrine, epinephrine, acetylcholine, tri-iodothyronine, and serotonin. Acetylcholine darkens *Fundulus* chromatophores but

lightens frog melanophores (121). The actions of the neurohumors reflect the origin of the melanocytes from the neural crest.

The most potent lightener of melanophores, studied by applying it to frog skin after darkening with melanocyte-stimulating hormone, is a new hormone, melatonin, which was isolated from cow pineal glands (127). The hormone has also been found in small amount in peripheral nerves of man, monkey, and cattle (128). The structure has been completely determined (129) and found to be *N*-acetyl-5-methoxytryptamine. An apparently unique enzyme system has been found which converts *N*-acetylserotonin, a serotonin metabolite, to melatonin (130). The role of melatonin in mammalian physiology has not yet been determined, but it is of interest that an extremely potent hormone affecting skin pigment cells has been isolated from the mysterious pineal gland.

Some progress has been made in the study of red hair pigments (131 to 134). The pheomelanins are yellow to red pigments which occur within the melanocyte as cytoplasmic granules, which under the electron microscope differ from eumelanin granules. The origin of the pheomelanin is not known, but a coupled oxidation of tryptophan metabolites with a reduction at a step in the tyrosine to melanin pathway is suggested.

It is usually assumed that the local function of melanin as far as the animal itself is concerned is to absorb light and protect from ultraviolet damage. The other effects have to do with camouflage and recognition. These have seemed insufficient explanations for a variety of reasons (97), including: (a) occurrence of melanin in structures below the epidermis is unexplained; (b) melanin tends to localize over the basal cell layer of the epidermis, but the site of primary events in sunburn is believed to occur in the prickly cells more superficial to this layer; (c) negro skin is more tolerant than white to chemicals and perhaps ionizing radiation as well as the ultraviolet; and (d) melanin has stable free-radical properties.

Some rationalization of the location of melanin between the basal cells and prickly cells is provided by the recent demonstration by Wheatley & Reinerstein (135) that the precursor of vitamin D is localized in the prickly cells and is not found in the surface lipid film as previously supposed. One can visualize the concentration of melanin over the basal layer as protecting the genetic material in this area by both optical and chemical means but rising higher in the epidermis as a sort of "melanin shutter" in response to the injury or the decreased need for the action of ultraviolet in providing energy for the molecular excitation and rearrangement of the vitamin D molecule. This energy is required for one of the few non-enzymic biochemical reactions.

Evidence now indicates that melanin has stable free-radical and semiconductor properties (136, 137). It probably serves therefore not only as a light filter but also as an electron trap and hence a physical-chemical protective device from the effects of ultraviolet and other radiation. Its functions in living organisms may be much broader than previously supposed.

## INFORMATION BARRIER AND TRANSDUCER

This classification overlaps, of course, with energy transfer. The information may cover a range of energies from that producing pain in a thermal burn to the lightest tickle. The report of the CIBA Foundation Study Group on Pain and Itch covers much of the information available in 1959 (8). In addition to the previous review (3), Shelley & Arthur have summarized their evidence for the role of proteolytic enzymes (endopeptidases) in pruritus (138, 139).

We shall attempt to summarize Weddell's findings (140 to 143) briefly in the following rules of skin sensation. There is an organization of nerve endings around hairs and in analogous positions in hairless areas. These are apparently concerned exclusively with the sense of touch. Between these endings there are fine, branched, naked axoplasmic filaments which subserve itch, pain, and temperature and can also act as touch receptors. These named sensory modalities used in the language of description do not necessarily correspond to specific nerve endings or specific tracts, but are part of a continuous series of responses. Each dorsal root neuron branches widely and innervates multiple areas of the skin surface or multiple hairs. Each hair or each skin area is innervated by branchings from multiple dorsal root neurons. Specific morphologic nerve endings and nerve fibers are not required for each sensory modality because a spectrum of information is transmitted in a spatial and temporal code. Temporal coding is accomplished in part by the variety, apparently random, of path-lengths of terminal arborizations serving any given area. The interpretations of Weddell and his group at Oxford have continued to be verified and expanded with greater correlation of the anatomical, electrophysiological, and subjective approaches to general sensitivity of the skin and cornea.

It is not the purpose of this article to review skin sensation in any completeness. However, the findings which have been summarized above represent a considerable conceptual change from older teachings about sensation of the skin and will undoubtedly lead to many new theoretical and practical considerations in the future.

Weddell and co-workers (8, 140 to 143) have demonstrated that many of the older interpretations of specific end bulbs were based on naming the variable contortions of the ends of regenerating nerves. The innervation of the skin is now considered to be plastic with constant degeneration and constant repair. Some of the other differences in interpretations between other workers and Weddell's group can be resolved if one assumes that the physical stimuli applied to the skin do not fire the nerve endings directly, but represent stimuli or injuries which produce tissue changes in cells in the vicinity of the nerve ending which then are transduced into a pattern of impulses. For example, release of intracellular potassium is said to be the stimulus to pain (144). Not everyone is ready to agree with all of Weddell's interpretations, but his findings do release the physiologists from the need for specific anatomical structures to explain the sensory modalities. For some time, the



psychologists have not been bound by this conceptual restriction (145).

Békésy (146) has summarized his fascinating investigations on similarities between hearing and skin sensations. After outlining the history of neglect of skin as an organ of sensation, he goes on to describe a surprisingly large number of similarities between hearing and the sensation of vibration along the skin. The apparent size of the sensation for sinusoidal stimulation decreases as the frequency increases. When the forearm is used as the basilar membrane in a model of the cochlea, although traveling waves move along the entire forearm, sensation is localized to an area of about two centimeters. The position of this sensation is frequency dependent. Directional hearing produced by a time difference in auditory stimuli has an analogy with time-separated vibrations applied to the thumbs. With the use of two microphones to produce vibrations in two cochlear models, it became possible for the subject to follow a sound source and even to project the source into the room. The sensations produced by the vibrations from rotating vibrators actuated by a rotating phase shifter were also projected outside the skin. This and several other phenomena are explicable in terms of inhibition. For example, if five vibrators of 20, 40, 80, 160, and 320 cycles per second are applied to the skin in a row, only the center one is felt. This funneling action of skin sensation is compared to the Mach ring, or law of contrast in vision. Inhibition between vibrators of different types can even produce a sensation of nothingness, a decrease in the spontaneous sensation level from the skin.

Mowbray & Gebhard (147) have also compared the ear and skin sensation, and point out the problem of explaining receptor mechanisms which can distinguish between rates in the vicinity of 350 cycles per second with less than 10 per cent error. Vibration can be sensed by the skin up to at least 1500 cycles per second and perhaps up to 10,000 per second.

Geldard (148) has pointed out the great neglect of the skin as a channel for the systematic input of information. He points out that the human integument rivals the ear as a temporal discriminator and shares with the retina the property of somewhat orderly spatial expansion. The skin has good "break-in" sensibility and may, therefore, be suitable for applications requiring warnings and alerts. He discusses a number of approaches to communication devices using the skin.

Sensory deprivation studies have suggested that the constant, "normal", virtually unconscious input of skin stimuli to the central nervous system is probably an important factor in the normal behavior and integration of the human organism (149).

The galvanic skin reflex has been widely used as a device for getting information out of the skin about people and their reactions. There is an extensive literature which has been the subject of reviews (150, 151).

#### REGULATORY MECHANISMS IN THE SKIN

The physiological relationships between the skin and the rest of the organism have been studied, particularly in relation to nerve function and

blood supply. With the exception of the axon reflex, now controversial (152), less attention has been paid to local controlling factors within the skin itself.

The neurohumors, vasoactive mediators, and the chemical mediators of itch and inflammation are a fascinating topic which is being covered in another review by Herxheimer (14). It is expected that these mediators of change from the normal state will receive increasing study and produce continuing new discoveries. In addition to these active mediators, we expect progress in the study of feedback mediators and feedback regulation of skin functions. If the stratum corneum is stripped off with tape, a vascular flare occurs and within five minutes changes begin in the basal cell layer which lead through increased mitotic activity to repair of the epidermal defect (153). Restripping of the surface, before a certain amount of stratum corneum repair, leads to no interruption of the repair cycle. With more extensive regrowth a new cycle of repair can be initiated (42). That these findings can be attributed to removal of something which feeds back to indicate intactness of the surface is shown almost conclusively by the findings of Bullough & Laurence (154) on control of epidermal mitotic activity in the mouse. They found that removal of epidermis produced a gradient of mitotic activity consistent with the diffusion from normal epidermis of a mitotic inhibitor. These findings are consistent with the interpretations of growth regulation in general advanced by Osgood (155). The stratum corneum, the so-called dead cell layer, is turning out to have many more functions than that of the mechanical protection, to which role its lack of nuclei has long consigned it. Analogy of these cells to the heirless but oxygen-bearing erythrocytes is immediately suggested. Lewis *et al.* (156) have reported with apparent surprise a smooth-muscle-stimulating, water-soluble material from the "dead" stratum corneum, and Pinkus (157) has discussed the interactions of the several cell types making up the epidermis in terms of a concept of symbiosis.

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## ADENOHYPOPHYSIS AND ADRENAL CORTEX<sup>1,2</sup>

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This review of the functions of the adrenal cortex and the adeno-hypophysis will be concerned mainly with the control of secretions from these glands and the mode of action of their hormones, with emphasis on the pertinent aspects of human physiology and patho-physiology. Although therapeutic aspects of ACTH and adrenal corticosteroids will not be discussed, as such, attention is called to a symposium on the subject of anti-inflammatory steroids by Bunim and others (28). Further reference is made at the outset to previous reviews on regulation of ACTH secretion, localization of hormones, hypothalamic-hypophysial relationship (71, 185, 189), and on steroid hormone metabolism, discussed by Katzman, Doisy & Matschiner (110).

### METABOLISM AND PHYSIOLOGICAL EFFECTS OF ACTH

Meakin *et al.* (142) found that the half life of ACTH in normal men varied from 4 to 18 min. and that it was not different in patients with Cushing's syndrome or Addison's disease. Initial levels were always higher with ACTH "A" than with ACTH "B", and ACTH "A" is more steroidogenic than "B" in humans, dogs, and rats. The short half life corresponds to a previous finding that a suboptimal intravenous dose of ACTH given to a hypophysectomized dog results in steroid output which lasts 15 to 30 min. (158). Adrenocorticotrophic hormone has been found to be resistant to purified thyroid and adrenal proteinases but is destroyed by trypsin and more slowly by pepsin (211).

Porcine ACTH is stable in 0.9 per cent saline at 20 to 37°C. at pH 2 to 7 but is inactivated by dog blood plasma or serum at 37°C. in 4 hr. as determined by Meakin, Tingey & Nelson (144). It is stable at 100°C. and pH 1 to 2 for 1 hr. Human ACTH activity is destroyed at 37°C. but appears stable for five to six months in the frozen state. The ACTH-inactivating system is destroyed by heating to 60°C. for 1 hr. Heat inactivation is inhibited by L-cysteine (143). In order to circumvent inactivation of ACTH, continuous infusion has been resorted to. By the intravenous infusion of a dose of only 0.8 to 1.4 U ACTH per 24 hr. in normal subjects, Nugent *et al.* (160) maintained plasma levels of free 17-hydroxycorticosteroids above the maximal normal level and obliterated the diurnal variation of plasma steroids. There-

<sup>1</sup> The survey of the literature pertaining to this review was concluded in May 1960.

<sup>2</sup> Among the abbreviations used in this chapter are: DOC (deoxycorticosterone); DOCA (deoxycorticosterone acetate); SC 8109 (a spiro lactone [Searle]); SU 4885 (2-methyl-1,2-bis(3-pyridyl)-1-propanone); TPN (triphosphopyridine nucleotide); and TPNH (triphosphopyridine nucleotide [reduced form]).



fore, it is understandable why it is so difficult to measure ACTH in human blood or plasma even during periods of maximal stimulation. McFarland, Clegg & Ganong (133) found the ACTH concentration in cavernous sinus blood of unanesthetized sheep to be twice that found in peripheral venous blood.

After intravenous infusion of  $^{131}\text{I}$ -labeled ACTH, the major part of the radioactivity was present in the kidney 20 min. after the injection [Cats *et al.* (30)]. Some of this was re-excreted into the blood stream; very little  $^{131}\text{I}$  ACTH appeared in the urine.

A number of additional cases of Cushing's syndrome with pituitary chromophobe tumor have been reported (86, 137, 159, 186). The largest single series, which was reported by Salassa *et al.* (186), was composed of 14 patients out of 122 with adrenal cortical hyperplasia. Their cases are important because some of the patients had symptoms and signs of pituitary tumor before adrenalectomy was performed; others had distinctly enlarged sella turcica before adrenalectomy. The unusual features were excessive pigmentation of the skin, unusual and severe eye difficulties, and very high levels of ACTH in plasma in the instances where measurement was made. The possibility that adrenalectomy activates the pituitary tumor is very real, but the occurrence of spontaneous tumors makes it clear that adrenalectomy is important in pathogenesis or acceleration in only a fraction of the cases. Nelson *et al.* (159) reported an average of three years from adrenalectomy to the development of the syndrome with a range of one to eight years. The ACTH measurements were made only in postoperative patients.

A somewhat related subject is the development of Cushing's syndrome in patients with tumor of another organ. Most patients have had small-cell carcinoma of the lung, but a variety of other tumors have been reported. A patient with carcinoma of the prostate was reported and the whole subject reviewed by Webster *et al.* (224), a patient with islet cell tumor of the pancreas with Cushing's syndrome was reported by Balls *et al.* (9), and a patient with carcinoma of the thyroid was reported by Dyson (55).

The importance of clinical recognition of the pituitary tumors in patients with Cushing's syndrome and Cushing's syndrome in association with malignant neoplasms elsewhere is obvious, but the implications of the pathological physiology involved are even more important and intriguing.

Emberland (61) showed that ACTH preparations are capable of stimulating the phosphorylase activity of fresh beef adrenal extract. The effect disappeared when the extract was heated, purified, or allowed to age. 5-Adenosine monophosphate has no influence on the phosphorylase activity of fresh beef adrenal extract but has a stimulating effect when the preparation is dialyzed. Two phosphorylase fractions were separable from the original extract. One of these was influenced more by 5-adenosinemonophosphate than the other but neither was influenced by corticotropin. Last year Haynes (90) and Haynes, Koritz & Peron (91) showed that ACTH caused an accumulation of adenosine-3'-5'-phosphate in incubates of beef adrenal cortex. This,

in turn, stimulated phosphorylase production. Adenosine-3'-5'-phosphate added to adrenal glands *in vitro* resulted in production of corticosteroids equal to or greater than that produced by ACTH.

Cohen (35) found that the glucose-6-phosphate dehydrogenase system was very active in fascicular and reticular zones of the adrenal gland; activity in the zona glomerulosa was comparatively low. This enzyme may be indirectly influenced by ACTH and is a potential provider of reduced triphosphopyridine nucleotide, which is a reductant highly important in steroid synthesis.

Attempts are being made to quantitate pituitary hormones by immunologic means. Fishman *et al.* (65) produced antibodies to thyroid-stimulating hormone, ACTH, and growth hormone in rabbits and then tested by means of the hemagglutination test in which the antigen is coupled to erythrocytes by means of diazotized benzidine. The antibodies to thyroid-stimulating hormone appear to have many cross reactions with other pituitary hormones. Antibodies to ACTH appeared to be hormone specific but not species specific, and growth hormone appeared to be species and, for most part, hormone specific.

Steelman & Smith (207) found ACTH highly adipokinetic whether the ACTH is pepsin-degraded or nondegraded. Alpha- and  $\beta$ -melanocyte-stimulating hormone are largely devoid of this activity. Lopez, White & Engel (128) found that, when adipose tissue is incubated in the presence of albumin and ACTH or epinephrine, fatty acids accumulate. Depletion of calcium abolished the lipolytic action of ACTH but not that of epinephrine. Adrenocorticotropin activity was restored by calcium ions but not by magnesium or potassium. Glucose inhibited action of both hormones. White & Engel (225) used both purified growth hormone and ACTH to stimulate lipid mobilization from the epididymal fat pad of fasting mice. Cortisol failed to induce lipid loss in intact mice and actually inhibited fat mobilization in adrenalectomized mice; the inhibition could be overcome by the simultaneous administration of ACTH. Purified ACTH is known to produce acutely fatty liver and ketonemia in rats and mice. Rudman *et al.* (183) were able to isolate a highly purified fraction from hog pituitaries free of other pituitary hormones; this final fraction has high lipemia-producing activity in the rabbit.

There have been widely varying results from studies of steroid and ACTH effects in intact animals and in man. Rosenfeld *et al.* (180) gave three injections of 40 units of ACTH gel at three- to twelve-day intervals and significantly enhanced elevated serum cholesterol and phospholipid levels in dogs which were subsequently fed cholesterol and thiouracil. Four of ten dogs developed paralytic stroke, and more severe atherosclerosis was found in the animals pretreated with ACTH. Skanse *et al.* (199) treated 25 patients with ACTH, prednisone, or cortisone for 10 to 49 days. Initially, total lipids, cholesterol, phospholipids, and  $\alpha$ - and  $\beta$ -lipoproteins in the plasma decreased, but this was followed by an increase in all fractions. On withdrawal, the concentrations of all lipid fractions rose. The differences in these results from

those reported by others were ascribed to length of treatment and time of sampling. It is significant that lipid levels rose.

On incubating rat adipose tissue *in vitro* in albumin-containing media, Orth, Odell & Williams (164) found that ACTH, growth hormone, epinephrine, and glucagon all increased oxygen consumption, decreased incorporation of acetate into lipid, and increased the amount of newly synthesized lipid attached to albumin. On the other hand, insulin increased oxygen consumption but increased incorporation of acetate into lipid and decreased binding of lipid to albumin; ACTH increased tissue non-esterified fat. The similar action of ACTH, growth hormone, epinephrine, and glucagon may be through entirely different mechanisms, but it is clear that they stimulate metabolic activity of adipose tissue.

Steelman & Guillemin (206) studied several highly purified  $\alpha$ -melanocyte-stimulating hormone preparations and demonstrated ACTH activity in them both by *in vivo* and *in vitro* assay:  $\beta$ -melanocyte-stimulating hormone did not exhibit any ACTH activity. The  $\alpha$ -melanocyte-stimulating hormone is a tridecapeptide with the same amino acid sequence as corticotropin A except that the N-terminal amino acid (serine) is acetylated and the C-terminal valine is in amide form. It has no effect on liver fat in a concentration 1000 times that of ACTH which is effective.

#### CONTROL OF ACTH RELEASE

Inasmuch as extensive reviews of the ACTH release have been reported (71, 185, 189), only brief discussion is indicated at this time. Corticotropin-releasing factor (CRF), oxytocin, and vasopressin are a family of peptides found in the neurohypophysis. All have the property of releasing ACTH, but corticotropin-releasing factor is much more potent (184); each one has some of the activities of the other two. Arginine vasopressin causes release of ACTH when a minute amount is injected into ventricle of the dog brain (120). Lysine vasopressin obtained from commercial powder causes ACTH release in the guinea pig (196). ACTH release in response to stimuli is abolished by destruction of the base of the hypothalamus and especially the median eminence. Royce & Sayers (182) purified a crude extract from calf brain, using carboxymethylcellulose columns, yielding a preparation that is briefly active in ACTH release but does not have ACTH or pressor activities. McCann & Haberland (129) obtained extracts of the pituitary stalk and median eminence areas of beef or rat brain. These extracts produced specific release of ACTH in animals with hypothalamic lesions, but 10 to 20 per cent of the activity appeared to be caused by vasopressin. Guillemin & Schally (82) demonstrated in an *in vitro* assay for corticotropin-releasing factor that there is no detectable destruction of ACTH and that it has no intrinsic ACTH activity or potentiating action. Dear & Guillemin (47) found an exponential decrease in sensitivity of the adrenal to ACTH with time after hypophysectomy or production of lesions in the median eminence. This decrease occurs despite large adrenals in animals with hypothalamic lesions; therefore,

animals with acute lesions only can be used for corticotropin-releasing factor assay. Midbrain section prevented ACTH release in cats in response to ether anesthesia and surgery (140).

Rinne *et al.* (174) found that synthetic oxytocin caused significant adrenal ascorbic acid depletion in normal animals but caused no depletion in animals blocked by prednisone. Furthermore, daily administration of oxytocin for six to nine days no longer caused ascorbic acid depletion. They concluded that oxytocin is not a specific corticotropin-releasing factor. Later, Kivalo & Rinne (112) used cortisone and dehydration to block ACTH release and stimulate antidiuretic hormone secretion and to observe the histological effects of these. They concluded that the activity of the cells of the supraoptic and paraventricular nuclei and release of ACTH did not have any connection but that the neurosecretory material around the region of the median eminence and hypophyseal portal vessels was related to ACTH release.

Hilton (97) studied the effect of various polypeptide hormones on the secretion of steroids by the isolated adrenal glands of hypophysectomized dogs. The 17-hydroxycorticosteroid in the adrenal vein effluent was measured and was specifically identified as cortisol. Synthetic lysine, arginine, and acetyl-arginine vasopressin were all active, but arginine vasopressin was the most active in stimulating cortisol production. In a dose of 0.4 U. per min., arginine vasopressin was as potent as ACTH. Oxytocin, insulin, glucagon, and pressor amines, such as epinephrine and norepinephrine, did not stimulate cortisol production. When perfused through isolated adrenals of hypophysectomized dogs, ACTH was found to stimulate secretion of aldosterone as well as cortisol. The role of arginine vasopressin as an important factor in adrenal response to stress was postulated. One of the most exciting aspects of these experiments was the perfusion of the adrenal gland preparation with adenosine-3'-5'-phosphate; this consistently produced cortisol in amounts equal to ACTH stimulus. Arginine vasopressin added to adenosine-3'-5'-phosphate enhanced cortisol production as long as it was being perfused but didn't have the more prolonged effect of adenosine-3'-5'-phosphate or ACTH. These data support and extend the experiments of Haynes and his co-workers (90, 91).

The effects of psychic factors, sedatives, tranquilizers, and anesthetics on ACTH release continue to be studied. Persky *et al.* (166) studied a group of very anxious people and found that the plasma levels of both adrenal weight maintenance factor and ascorbic acid depletion factor were distinctly higher in the anxious patients than in controls. Plasma cortisol levels were also significantly high in anxious patients (85, 166). The response to both ACTH and typhoid vaccine was partially inhibited by large doses of chlorpromazine in schizophrenic subjects (67). Large doses of chlorpromazine given to rats produced a distinct increase of adrenal weight, but cortisol administered simultaneously prevented the effect (103). Meprobamate is able to inhibit ascorbic acid depletion caused by psychic stress. Prolonged administration of meprobamate, for 11 to 14 days, abolishes its blocking

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effect on ascorbic acid depletion caused by stress (134); there is no blocking of exogenous ACTH effect. Chlorpromazine and iproniazid, given orally in single doses, produce ascorbic acid depletion of the adrenal cortex but not of the liver. Since epinephrine and norepinephrine also decrease ascorbic acid, iproniazid may act by its monamine oxidase inhibiting action on these compounds (188).

Kitay *et al.* (111) found that acute ACTH release as measured by ascorbic acid depletion was stimulated by a single injection of epinephrine or reserpine, and prolonged hypersecretion of ACTH resulted from repeated injections. Response to ether anesthesia was partially diminished or was absent after a single injection of epinephrine or multiple injections of reserpine, but response to ACTH was unimpaired. An ingenious experiment was devised by Suzuki *et al.* (209) to obtain evidence for the effect of ether anesthesia. Preliminary dorsal rhizotomy from the eleventh thoracic through the third lumbar nerves was performed to permit the obtaining of blood from the intact adrenal vein without pain. There was a striking increase in the 17-hydroxycorticosteroid secretion after 30 to 60 min. of ether anesthesia.

Thiouracil fed to rats produced adrenal atrophy and enlargement of the thyroid and pituitary (123). The pituitary content of ACTH was reduced to one-third that of controls.

Brown *et al.* (26, 27) obtained subcellular fragments of the adenohypophysis by means of differential centrifugation: ACTH was found in the microsomal and supernatant fractions, thyroid-stimulating hormone in the mitochondria, luteinizing and growth hormones in the acidophilic granules, and follicle-stimulating hormone in multiple fractions. It has been possible for the first time to cultivate cells of the human pituitary and to isolate somatotropin, gonadotropin, and ACTH from these cells (210).

The exploration of the role of hypothalamic centers continues with the use of electrolytic lesions by stereotactic instruments or by ablation. Lesions of the median eminence in the rat block adrenal ascorbic acid depletion normally occurring 1 to 4 hr. after unilateral adrenalectomy. Three weeks after production of the lesion the testes are normal in size, but there is atrophy of the adrenal cortex and decrease to 50 per cent of normal ACTH content in the pituitary (130).

Yamada & Greer (233) found that bilateral obliteration of the amygdala in the male rat does not interfere with thyrotropic or corticotropic secretion by the pituitary. Stimulation of the amygdala in primates has resulted in marked increase of the 17-hydroxycorticosteroid content of serum (141). Slusher & Critchlow (201) produced coagulation lesions of the posterior portion of the midbrain at the level of rostral pons in rats which resulted in increased corticosterone secretion into the adrenal vein, whereas lesions in the diencephalon and rostral midbrain resulted in depressed secretion. Curiously enough the adrenal ascorbic acid response was not correlated with corticosterone response to ACTH. They concluded that there was no extrahypothalamic source for corticotropin-releasing factor. On the other



hand, Egdaahl (59) and Hume & Egdaahl (104) performed a large number of brain extirpation experiments in dogs and found that removal of the brain above the superior colliculus leaving the pituitary *in situ* resulted in a high resting corticoid level, probably attributable to removal of cortical inhibitory effects. Two-thirds of the animals responded to burn trauma with increased corticoid output. Their experiments can be interpreted best by postulating a hindbrain factor which causes ACTH release to account for response to trauma. The difference in results could be species difference or could arise because more precise lesions are possible in the dog than the rat because of size. Removal of central nervous system inhibiting factors is evident also in the studies of Gellert & Ganong (73) in rats with electrolytic lesions. Lesions in the arcuate nucleus in the posterior tuberal region of the hypothalamus resulted in early maturation and estrus, whereas lesions in the anterior hypothalamus, mammillary body, hippocampus, cortex, and thalamus had no effect on maturation.

Moll *et al.* (152) produced electrolytic lesions at 20 regularly distributed points in the middle of rat hypothalamus. Eighteen days later the first adrenal was removed and six days later the other adrenal. With lesions in the anterobasal and midbasal hypothalamus, the rats failed to respond with compensatory adrenal hypertrophy, whereas lesions of the pituitary stalk and median eminence had no effect on adrenal weight. These findings appear to be paradoxical unless the higher lesions interrupted some nervous pathways. Moll ascribes the difference to the size of his lesions and to the time interval between lesions and determination of adrenal weight. Smelik *et al.* (202) found exactly the opposite results, and their findings correspond with the majority of observations. The localization of vital functions to very small and specific areas, of course, is of great importance in understanding the complex role that the central nervous system has on the function of the pituitary.

Martini *et al.* (139) grafted anterior pituitary tissue into the anterior chamber of the eye. There were no evident growth hormone or gonadotropic activities. Thyrotropic and corticotropic activities were indicated by partial maintenance of thyroid and adrenal weight, ACTH response to Pitressin, lysine vasopressin, and Guillemin's fraction D, <sup>131</sup>I studies, and signs of physical and chemical activity of the glands. Subnormal response is probably related to anatomical separation from the hypothalamus and the portal hypophyseal vessels. An autograft of the adrenal can be stimulated by large doses of ACTH; nevertheless, the regeneration is less than occurs in rats with normal pituitaries (170).

#### MEASUREMENT OF ACTH PRODUCTION *IN VIVO*

One of the most significant developments has been an expansion of the use of an 11 $\beta$ -hydroxylase inhibitor, 2 methyl-1,2-bis(3-pyridyl)-1-propanone (SU 4885) as a test substance for study of ACTH release in man and in animals. It interferes with cortisol, corticosterone, and aldosterone production. The most extensive study is that of Liddle *et al.* (124). When SU 4885 was

given orally, there was a sharp rise in plasma and urine 17-hydroxycorticosteroids in normal persons and in patients with a variety of illnesses including myxedema. There was no response in patients with hypopituitarism from any cause including pituitary irradiation. Of course, there was no response in patients with Addison's disease. When SU 4885 was injected intravenously, the response was similar. Bissell *et al.* (17) and Gold *et al.* (75) made the important observations that 0.5 mg. of 9 $\alpha$ -fluorohydrocortisone given intravenously at the end of an infusion of SU 4885 blocks completely the rise of plasma 17-hydrocorticosteroids or increase in excretion of 17-ketogenic steroids and interrupts a response that is started.

Nelson, Meakin & Thorn (159) made a direct measurement of plasma ACTH in patients with Addison's disease and demonstrated a striking decrease after cortisol infusion. The block by 9 $\alpha$ -fluorohydrocortisone and the plasma ACTH changes indicate the striking sensitivity of the central nervous system to circulating corticosteroid and strengthens the evidence for a feedback mechanism in the control of ACTH release. The evidence obtained from use of the inhibitor SU 4885 and 9 $\alpha$ -fluorohydrocortisone is of great importance, since it occurs in normal individuals who have not been subjected to any serious stressful procedure. The evidence is indirect, as the response is measured by lack of excretion of adrenal steroids. The direct ACTH measurement is equally important, to be sure, in patients with adrenal or pituitary disease. The question raised by Ganong & Forsham (71) about the factors responsible for ACTH release during acute stressful circumstances is not answered by these studies.

Von Froesch *et al.* (221) administered SU 4885 to a patient with adenoma of the adrenal cortex. Although the patient had a striking response to ACTH, there was no response to SU 4885. The adenoma apparently was autonomous in that it produced a constantly greater than normal cortisol supply and apparently caused unresponsiveness of the mechanisms controlling ACTH release or production or both. The adenoma nevertheless was very responsive to exogenous ACTH.

Deoxycorticosterone and 11-deoxycortisol are excreted under the influence of endogenous ACTH stimulus. When prednisone is used to suppress ACTH production, it is possible to inhibit aldosterone secretion and therefore to influence sodium excretion in secondary aldosteronism (38). Such a combination of therapy has obvious therapeutic implications.

Gold (74) measured the biologic half life of 11-deoxycortisol in a variety of clinical conditions: it is 42 min. in normals, but it may be prolonged to as much as 80 min. in liver disease or 108 min. in hypothyroidism; it is approximately half that of cortisol. Other amphenone compounds will be discussed below in relation to steroidogenesis.

By paper chromatography, using a methylcyclohexane: dimethylformamide solvent system, Touchstone *et al.* (212) found 11-deoxycortisol in a concentration about one-tenth that of cortisol in the adrenal venous blood of nine hypertensive and one normotensive patient undergoing adrenalectomy.

Therefore, 11-deoxycortisol must be considered a normal but minor secretory product of the adrenal cortex.

Five of six patients with suprasellar tumors tested by Hökfelt & Luft (98) responded to SU 4885 administration. Six of eight patients with suprasellar tumors excreted aldosterone but did not increase excretion in response to salt restriction. Hökfelt & Luft also found a loss of diurnal rhythm of 17-hydroxycorticosteroid excretion in four of seven patients tested. Loss of adrenal cortical periodicity has also been found in a group of five patients with adrenal cortical hyperplasia with Cushing's syndrome (53).

The problem of adrenal and pituitary responsiveness after ACTH or steroid therapy has been the focus of much attention. Holub *et al.* (102) made direct measurement of pituitary ACTH in patients who had large doses of steroid until time of death. One acute leukemia patient had ten months of cortisone therapy at an average dose level of 380 mg. of cortisone-equivalent. This patient's pituitary gland had Crooke's changes and had 30 per cent of the normal ACTH concentration. Four other patients, who had had either shorter treatment or a smaller daily dose, had normal ACTH concentrations. Four patients were tested with SU 4885 after varying periods of steroid therapy measured as cortisone-equivalent: 24 days with 2000 mg. per day, 5 years with average of 50 mg. per day, 3 years with average of 25 mg. per day, and 10 years with an average of 100 mg. per day. Repository ACTH was given for two to four days with an interval of three days before SU 4885 testing. The response was barely subnormal in the second patient, normal in patients one and three and absent in the fourth patient. Therefore, a majority of patients respond normally to stimulation of the pituitary or give direct evidence of normal ACTH concentration in the pituitary. There are a few patients who fail to respond, such as the ones reported by Kyle *et al.* (121). Armatruda *et al.* (1) found normal adrenal cortical responsiveness to insulin-induced hypoglycemia with normal rise in plasma cortisol. It is noteworthy that these investigators continued every-other-day injections of 40 units of zinc ACTH for the period of tapering the use of prednisone and for four additional days after stopping it. In effect, there was a sudden withdrawal of higher than normal circulating cortisol when ACTH was stopped, so withdrawal symptoms were to be expected.

Holub *et al.* (101) examined rat pituitaries after chronic cortisone administration and demonstrated a persistence of adrenal atrophy in spite of partial repletion of pituitary ACTH after ten days, suggesting that ACTH release is negligible. Simultaneous ACTH administration did not modify the striking reduction in ACTH release after stress, but, of course, ACTH prevented adrenal atrophy.

Marks *et al.* (136) tested responsiveness of patients to intravenously administered ACTH after prolonged corticosteroid therapy (from four months to seven years). Two patients received ACTH therapy for three weeks and three months, respectively. It is quite difficult to judge the response of these patients to operation, because in seven of nine operations the patients

with steroid therapy received a significant dose of corticosteroid before operation. There is also a great difference between types of operation. The most important question, however, relates to the discrepancy in duration of previous therapy between the two groups. The importance of duration as well as total dosage has been discussed above (102).

Felber *et al.* (64) studied the adrenal responsiveness in twelve patients with hyperthyroidism and nine with myxedema. In hyperthyroidism the response to ACTH was normal on the first day, but there was no increase on the second day. Some patients with myxedema had a subnormal response to ACTH on two days, but others had a supernormal response on the second day. Therapy of myxedema tended to result in the return of response to normal. Liddle *et al.* (124) found the responsiveness of myxedema patients to SU 4885 to be normal.

In a group of ten women with anorexia nervosa, Fletcher & Brown (66) found normal responsiveness to ACTH. Liddle *et al.* (124) found that two anorexia nervosa patients were unresponsive to SU 4885. They also found unresponsiveness to SU 4885 after cessation of both corticosteroids and ACTH.

Melby (145) found an accelerated disappearance rate of cortisol in hyperthyroidism and in patients given tri-iodothyronine. The hydroxycorticosteroid excretion measured as Porter-Silber chromogens accounted for a smaller than normal portion of the 17-ketogenic steroids excreted by these persons; the major components were metabolites with reduction at C-20. This finding indicates a need for caution in the interpretation of responsiveness and total metabolism of cortisol on the basis of Porter-Silber chromogens alone in hyperthyroidism. Romanoff *et al.* (178) found a constant rate of cortisol and cortolone production in normal adults at all ages. It should be noted that pregnancy, estrogen administration, neonatal metabolism, liver disease, and familial nonhemolytic jaundice all introduce variable production of 17 and 21-hydroxy, and 20-ketometabolites of cortisol and, therefore, caution in interpretation is needed just as in hyperthyroidism (146, 175, 214, 217).

#### STEROIDOGENESIS

Bergental *et al.* (13) found the active agent in technical dichlorophenyl dichloroethane (DDD) to be 2,2-bis(4-chlorophenyl,2-chlorophenyl)-1,1-dichloroethane (*o,p'* DDD). It causes atrophy of the zona reticularis and fasciculata of the adrenal cortex. The agent is capable of producing regression of metastatic cancer of the adrenal cortex and inhibiting hormonal secretion, as well as inhibiting normal adrenal cortex. In another study by Vilar & Tullner (219) with this compound, the adrenals of dogs were examined two, four, and six days after starting the drug, and focal degenerative lesions were found in the zona reticularis but no changes in the zona glomerulosa.

Amphenone B was given to intact and hypophysectomized frogs (52). There was marked hypertrophy of the interrenal gland, and distinct changes

occurred in the lipids whose granules became irregular and of a variable size, filling up the cytoplasm of the cells. If the brain is extirpated, amphenone did not produce any appreciable changes in the adrenal.

Tullner (213) used a compound related to amphenone, methylenedianiline, which inhibits 17-hydroxycorticoid excretion in the dog. In rabbits this compound elicits a progestational proliferation of endometrium, but this does not occur in adrenalectomized or corticoid treated rats; therefore, it is adrenal dependent. This compound does not have the methyl and ketone group of amphenone and yet it has similar biological effects.

Edgren *et al.* (57) produced adrenal atrophy in rats by the administration of 6 $\alpha$ -methyl-17-acetoxypregesterone, and the animals responded poorly to cold shock. This substance apparently suppresses ACTH secretion although it is ineffectual as a glucocorticoid.

The naturally occurring blocks of steroidogenesis and of steroid metabolism continue to reveal important information about this subject. In a study of adrenal hyperplasia, Hill (94) fractionated the 17-ketogenic steroids by means of elution from a silica gel column and found a ratio of 11-deoxycorticosteroids to 11-oxycorticosteroids of 2.3 compared with 0.24 in normals. In the case of a nine-year-old boy with congenital adrenal hyperplasia, reported by Chaptal *et al.* (34), 11-deoxycortisol and its tetrahydro derivative were found in the urine; this signifies absence of 11 $\beta$ -hydroxylase. The boy was normotensive. Bongiovanni *et al.* (21) found excretion of an average of 1.17 mg. C-21 methyl corticosteroids per kg. per day in 11 patients with congenital adrenal hyperplasia, compared with 0.01 mg. per kg. per day in normals and 0.07 mg. per kg. per day in patients with virilizing adrenal tumors. Cox (42) devised a method for quantitative determination of this group of acetaldehydogenic steroids with as small an amount as 2.0  $\mu$ g.

The steroid or steroids responsible for virilization in congenital adrenal hyperplasia have not been determined. Since large amounts of 17 $\alpha$ -hydroxypregesterone are secreted in all cases of congenital adrenal hyperplasia, this substance has been incriminated in the past. Axelrod & Goldzieher (5) administered 17 $\alpha$ -hydroxypregesterone to four subjects and determined the excretory products. They concluded that the metabolites of 17 $\alpha$ -hydroxypregesterone do not seem to account for the masculinization seen in congenital adrenal hyperplasia. Yet, from their table, the ratio of etiocholanolone to androsterone is about the same in the control period in one untreated patient with congenital adrenal hyperplasia as in the three patients with unrelated endocrine disorders after administration of 17 $\alpha$ -hydroxypregesterone. Solomon *et al.* (204) injected tritiated 17 $\alpha$ -hydroxypregnenolone into a subject with metastatic adrenal cancer. Radioactive dehydroisoandrosterone, androsterone, and etiocholanolone were isolated from the urine and accounted for 4.3 per cent of the administered steroid. It is interesting that 17 $\alpha$ -hydroxypregnenolone has been isolated from adrenal vein effluent in dogs, after giving ACTH, by Carstensen *et al.* (29). They suggest that this precursor is found because there is a saturation of enzyme systems necessary

for full conversion of these precursors to cortisol, etc. Bradlow & Gallagher (22) gave  $11\beta$ -hydroxy- $\Delta^4$ -androstene-3,17-dione to a patient with congenital adrenal hyperplasia and found three major metabolites found in normal persons.

Oertel & Eik-Nes (161) showed that dogs, which have a low circulating level of dehydroepiandrosterone, had very high levels after intravenous administration of pregnenolone and  $17\alpha$ -hydroxypregnenolone.

Some previously undescribed steroids have been found in the urine of a patient with adrenal carcinoma with metastases by Okado and co-workers (162);  $3\beta,7\alpha,16\alpha$ -trihydroxy- $\Delta^5$ -androstene-17-one and  $3\beta,16\alpha$ -dihydroxy- $\Delta^5$ -androstene-7,17-dione were isolated for the first time;  $3\beta,7\alpha$ -dihydroxy- $\Delta^5$ -androstene-17-one was isolated from the urine of a normal man given dehydroisoandrosterone orally, and  $3\beta,16\alpha$ -dihydroxy- $\Delta^5$ -androstene-17-one was isolated from the urine of a normal man.

Ulstrom & Colle (214) isolated and identified  $6\beta$ -hydroxycortisol and its reduced derivative from the urine of newborn infants in rather high quantity. It is not possible to conjugate  $6\beta$ -hydroxycortisol with glucuronide or sulfate at the 3 oxygen so it is excreted as free steroid. Neonatal infants do not appear to respond to ACTH during the first week of life, but it is possible that some still unidentified steroid is produced. Women in the last trimester of pregnancy excrete a greater amount of  $6\beta$ -hydroxycortisol than normal women.

Migeon (146) reviewed the subject of cortisol formation and metabolism in the neonatal period and reported his own studies using cortisol-4- $^{14}\text{C}$ . The most striking feature of metabolism of cortisol is the consistent finding of large amounts of metabolites not accounted for by known metabolites. This fraction was as high as 80 per cent of the radioactivity measured, compared with 25 per cent in adults.

A metabolite of prednisolone, 4-5-dihydroxyprednisolone, has been found to have distinct glucocorticoid activity, about half that of cortisone (72). The reduced metabolic products of cortisol and cortisone are inert. The rate of transformation of  $\Delta^1$ -steroids is considerably slower than cortisol or cortisone since ring A is less susceptible to hydrogenation. Vermeulen & Caspi (218) found larger amounts of free than conjugated steroids in leukemia patients and isolated a new compound  $11\beta,17\alpha,20\alpha$ -21-tetrahydroxy-1,4-pregnadien-3-one as well as two other C-20 hydroxy compounds.

Brown *et al.* (25) showed clearly that the adrenal cortex is a source of estrogens in oophorectomized women with breast cancer. When ACTH is given, there is a three- to ninefold increase in estrogen excretion. Adrenalectomy reduced the excretion of estrogen sharply.

Desaulles (51) studied the effects of  $3\beta,16\alpha$ -dihydroxyallopregnan-20-one from the urine of a patient with the salt-losing type of congenital adrenal hyperplasia. It had been isolated from the urine by Neher, Meystre & Wettstein (157). This substance promoted sodium loss in adrenalectomized rats when given in minute doses—0.2  $\mu\text{g}$ . per kg. to 1.0  $\mu\text{g}$ . per kg. Only 70 per cent of animals responded, however.

Rosemberg *et al.* (179) also have reported that a substance in the urine of patients with the salt-losing type of congenital adrenal hyperplasia promoted natriuresis and, in addition, found that it inhibited the sodium-retaining effect of deoxycorticosterone acetate in adrenalectomized rats. In their patient on a low sodium intake, plasma sodium fell, sodium excretion remained high, and aldosterone excretion rose to 18  $\mu$ g. per day. The substance had the characteristics of 17 $\alpha$ -hydroxypregnenolone. Cortisone caused the decrease of a high level of aldosterone excretion to normal in two subjects with congenital adrenal hyperplasia described by deGraeff & Moolenaar (48). Coppage & Liddle (39) administered 3 $\beta$ ,16 $\alpha$ -dihydroxy-allopregnan-20-one isolated from the urine of patients with salt-losing virilizing adrenal hyperplasia to five humans in doses up to 400 mg. per day but failed to modify electrolyte excretion. It would have been interesting if this compound were administered to infants or young children, since it is in the early years that salt-losing is a problem. It is just possible that the infantile and juvenile kidney would respond differently from that of an adult.

When ACTH was given to a patient with a salt-losing form of congenital adrenal hyperplasia, Jailer *et al.* (107) found a marked increase in urinary sodium and a decrease in serum sodium while the patient was receiving either 9 $\alpha$ -fluorohydrocortisone or deoxycorticosterone acetate. This finding is compatible with the idea that ACTH stimulates the adrenal cortex to secrete a steroid which either causes sodium excretion or, at least, antagonizes aldosterone just as it clearly antagonizes administered sodium-retaining hormones. The need for twice the usual dose of deoxycorticosterone acetate sufficient for adults with Addison's disease has suggested for a long time that the adrenal gland produces a salt-losing substance. On the other hand, Blizzard *et al.* (19) have studied three patients with the salt-losing form of congenital adrenal hyperplasia and have found very low levels of aldosterone production by bioassay, chromatographic-chemical, and isotope-derivative methods. They conclude that these patients are unable to synthesize adequate amounts of aldosterone even in the face of dehydration and hypovolemia. The role or even the secretion of a salt-losing steroid has not been clarified as yet. It is possible that there are several defects in the adrenal gland including failure to synthesize adequate amounts of aldosterone on the one hand and secretion of a salt-losing steroid on the other.

When Korus *et al.* (115) perfused the adrenal gland of a patient with Cushing's syndrome, the main products were cortisol and cortisone; 11 $\beta$ -hydroxyandrostenedione,  $\Delta^4$ -androstenedione, and androsterone were formed in lesser quantities. Deoxycorticosterone in the perfusate resulted mainly in secretion of corticosterone, but some 11-deoxycortisol was secreted; no estrogens were formed. Lombardo *et al.* (126) also found conversion of deoxycorticosterone to corticosterone when human adrenal glands were incubated *in vitro*. With other substances they found cortisol as the major product. They also studied the adrenal vein blood (127) of twelve breast cancer patients undergoing adrenalectomy. Cortisol was isolated in every instance, corticosterone in six instances, 11-deoxycortisol in five instances,



and 11 $\beta$ -hydroxy- $\Delta^4$ -hydroxyprogesterone in ten instances. Isolation of 17 $\alpha$ -hydroxyprogesterone in five samples and dehydroisoandrosterone in one sample is reported for the first time.

Bailey *et al.* (8) studied steroids produced by incubated human adrenal tissue obtained at operation for a variety of conditions. Adenomata from patients with hyperaldosteronism and nontumorous adrenal tissue from patients with idiopathic edema and gonadal dysgenesis produced cortisol, corticosterone, and aldosterone. Hyperplastic adrenals from patients with Cushing's syndrome produced cortisol, corticosterone, and, in one instance, aldosterone. Corticosterone and aldosterone were unusually increased after ACTH was added, but cortisol was consistently increased in all adrenals incubated when ACTH was added. Further *in vitro* incubation studies certainly will lead to more valuable information about both normal and pathological physiology.

Villee *et al.* (220) incubated homogenates of the fetal adrenal in the presence of a TPNH-generating system with progesterone or 17 $\alpha$ -hydroxyprogesterone as a substrate. At all ages, adrenal homogenates possessed ability to convert progesterone to yield Porter-Silber chromogens. They also found that mitochondria were more potent than the supernatant and that there was an active hexose monophosphate shunt which could generate TPNH for steroid hydroxylation. Schönbaum *et al.* (195) found that the effect of ACTH on the formation of steroids by the rat adrenal *in vitro* is greatly enhanced by glucose, adenosine, uridine, and TPNH. The effect of glucose is inhibited by 2-deoxyglucose, but the inhibition is overcome by TPNH. During incubation of adrenal fragments, but not intact glands, TPN-dependent dehydrogenases leak into the medium. In the presence of TPN, glucose-6-phosphate tremendously enhances steroid formation and is more effective than addition of TPNH alone, since the glucose-6-phosphate and TPN provide a continuous supply of TPNH. The availability of TPNH appears to be one of the important determinants of rate of steroid formation.

When rat adrenal homogenates were incubated with TPN and glucose-6-phosphate, Koritz & Péron (114) found that both calcium ion and freezing enhanced corticoid production. Versene inhibited stimulation by calcium but did not inhibit the freezing effect unless versene was added before freezing; calcium reverses the versene effect. Strontium, magnesium, barium, and ferrous iron are only half as effective as calcium in this system. The basic reaction may be proteolytic.

Van der Vies (216) measured the corticosteroid output of adrenals *in vitro* from rats with various lesions. The output was reduced after hypophysectomy, hypothalamus lesions, cortisol, and pentobarbital or morphine. Histamine or ACTH increased steroidogenesis. Daily treatment of the hypophysectomized rats with a potent ACTH preparation prevented the decreased steroid production and adrenal atrophy. Lysine vasopressin given to rats with hypothalamic lesions had a small but significant effect.

Fatty-acid-deficient diets fed to rats by Hayashida & Portman (88) re-

sulted in a decrease in unsaturated fats in the adrenal gland and an accumulation of cholesterol esterified with saturated and mono-unsaturated fatty acids. The adrenals of animals fed the fatty-acid-deficient diet secreted smaller quantities of steroid hormones *in vitro* under stimulation of ACTH than did controls.

The role of ascorbic acid in steroidogenesis is still not completely clarified. There is a great species difference. For instance, Schindler (192) found that the adrenal of the golden hamster secretes only cortisol, the level of cortisol is one-fourth to one-tenth that of other animals, and there is no cholesterol in the gland. Cholesterol is not converted to cortisol *in vivo* but is converted to cortisol and other compounds by hamster adrenal homogenates (193). Elton *et al.* (60) demonstrated that ACTH does not reduce ascorbic acid in adrenal glands of frogs, toads, chickens, rabbits, cats, or dogs, and cholesterol does not change in most species. In fact, adrenal cholesterol increases after cold exposure in rabbits and after ACTH in frogs. Brodish & Long (24) found that the rate of change of adrenal ascorbic acid in the rat is uniform and the depletion rate is 120 times as rapid as repletion. There is a linear relationship between dose and response up to 5  $\mu$ g. of ACTH at one hour. The dose response curve can be extended by using a 2 hr. interval after ACTH. Lipscomb & Nelson (125) followed the adrenal vein ascorbic acid in hypophysectomized rats and found that there is an initial sharp rise of ascorbic acid to at least twice the control level within 2 min. after small doses of ACTH, followed by an abrupt fall to levels lower than pre-ACTH ones by 10 min. A sharp rise in adrenal vein corticosterone begins as the ascorbic acid is falling.

Perič-Golla *et al.* (165) studied the loss of cortisol-C<sup>14</sup> in the adrenal of normal and ascorbic-acid-deficient guinea pigs and found that the rate of loss of labeled cortisol was more rapid than in normals and that this rapid loss was not found in plasma, liver, kidney, spleen, or thymus. This finding suggests that ascorbic acid may affect synthesis, exchange and, particularly, release of cortisol. It is fair to say that the role of ascorbic acid in steroidogenesis and metabolism is still obscure.

#### METABOLIC EFFECTS OF CORTICOSTERONE, CORTISOL, CORTISONE AND THEIR METABOLIC PRODUCTS

The metabolism of corticosterone in man has been studied by Peterson & Pierce (169). The disappearance rate was more rapid than cortisol with a half-time of 1 to 1.5 hr., was faster in hyperthyroidism, and was slower in cirrhosis and myxedema and after estrogen treatment. About 3 per cent is excreted unconjugated, and 40 to 60 per cent as metabolites hydrolyzed by  $\beta$ -glucuronidase. Corticosterone and especially its acetate are poorly absorbed from the gastrointestinal tract. The miscible pool size was only 0.2 to 0.4 mg. and there was a turnover of 0.7 mg. per hour. These data indicate that about one-fifth to one-tenth as much corticosterone as cortisol is produced. Peterson (167) also summarized the currently available data on half life and

turnover time of a number of corticosteroids. Willoughby *et al.* (228) injected corticosterone  $4C^{14}$  in dogs and demonstrated that the rate of removal is clearly diminished in hepatectomized animals and that there is a distinct enterohepatic circulation. Bojesen & Egense (20) showed that total evisceration eliminated all true elimination of corticosteroids, and the perfusion of the hindquarter resulted in no detectable elimination even during electrical nerve stimulation.

The intermediary metabolism of cortisol was extensively studied by Gold *et al.* (76) in volunteers. There is very little interconversion of the two major metabolites of cortisol; tetrahydrocortisone and tetrahydrocortisol are derived mainly from cortisone and cortisol respectively, and they are not converted to allotetrahydrocortisol. The three metabolites mentioned above account for 80 per cent of the turnover of cortisol. The ratio of tetrahydrocortisol to tetrahydrocortisone rises strikingly after trauma.

The effect of estrogens and pregnancy on plasma levels of cortisol introduces the subject of protein binding of cortisol. The evidence for increased binding of cortisol as a result of estrogen therapy and pregnancy is clearly established by the work of many investigators (36, 149, 168, 175, 187, 222). However, Herrmann *et al.* (92) gave 5.0 mg. premarin per day and found plasma levels not significantly affected, but on the fifth day of this therapy there was a diminished response to ACTH as measured by 17-ketogenic steroids.

Schedl *et al.* (191) determined the ultrafiltrable cortisol by using doubly labeled cortisol. About 10 per cent of total cortisol is ultrafiltrable, and 80 to 90 per cent of this is reabsorbed by the tubules. This means, of course, that tubular reabsorption of cortisol is not very important in the total economy of cortisol. However, Cope & Black (37) found that the excretion of unaltered cortisol was distinctly higher in patients with Cushing's syndrome and that measuring the urinary cortisol was the most discriminatory of all tests used for the diagnosis of Cushing's syndrome. This finding suggests an increased absolute level of unbound cortisol in Cushing's syndrome.

A significant fraction, averaging about 25 per cent, of the free 17-hydroxycorticosteroids of various blood samples was associated with red cells. Migeon *et al.* (147) conclude that the red cells appear to play a significant role in the transport of cortisol.

By means of equilibrium dialysis, Wallace & Carter (222) found the cortisol binding by protein to rise from 90 per cent to 97 per cent, and the plasma level to rise from 20 to 50  $\mu\text{g. per } 100 \text{ ml.}$  after estrogen administration. They found a delay in clearance of tetrahydrocortisol as well as of cortisol, contrary to the findings of Peterson *et al.* (168) that clearances of cortisol and corticosterone were delayed while those of cortisone, dihydro- and tetrahydrocortisol were unaffected. The urinary 17-hydroxycorticosteroids were decreased to 30 to 50 per cent of control levels, as found by others (122, 149, 175). The plasma corticoid levels rose to 40 to 50  $\mu\text{g. per } 100$

ml. and remained elevated for about a month after cessation of estrogens. Mills *et al.* (149) used ultrafiltration at 37°C. and a double isotope tracer and found results essentially in agreement with the others. The absolute amount of unbound cortisol remained the same when estrogens were administered in spite of a striking rise in total cortisol. At a temperature of 4°C. protein binding determined by their method increases, but at 37°C. their results agree with those of Wallace & Carter (222) carried out at 4°C.

Robertson *et al.* (175) postulate a metabolic block in the saturation of ring A of cortisol to account for the slower removal rate of cortisol after administration of estrogens. The evidence for this was that in a patient with Addison's disease urinary tetrahydrocortisol and tetrahydrocortisone increased less after an intravenous infusion of cortisol while the patient was receiving estrogens than during control period. The same finding was made in a normal patient given ACTH with and without estrogens. These findings have an important bearing on the demonstration by others (122, 168) of a decrease in 17-hydroxycorticosteroid excretion, interpreted as decreased cortisol production. Patients receiving estrogens for a long time do not exhibit Crooke's changes in the pituitary.

Venning *et al.* (217) found, in agreement with Robertson, that the ratio of the sum of tetrahydrocortisol plus tetrahydrocortisone to the sum of cortisol plus cortisone in pregnancy decreases from a normal mean of 8.10 to 4.08, and in two pregnant women with Addison's disease it decreased further to 2.0 and 0.7.

Bernstein (14) studied the effect of ACTH on adrenal glands transplanted into the portal circulation of the rat. Estrogens had a striking effect on the gland. The transplantation of the adrenal into the portal circulation of the female was associated with striking hypertrophy of the adrenal. Oophorectomy eliminated the hypertrophy, and male rats subjected to this transplant procedure did not manifest this change.

Idler *et al.* (106) found that female sockeye salmon have plasma cortisone and cortisol levels twice that of male salmon at time of spawning migration. This finding is similar to that found during pregnancy in mammals.

Sandberg & Slaunwhite (187) have found the plasma transcortin binding of cortisol-4-<sup>14</sup>C is low in infants, probably low in patients with nephrotic syndrome and in some patients with multiple myeloma, normal in patients with cirrhosis, and slightly increased in patients with rheumatoid arthritis. Estrogens cause greatly increased transcortin binding of cortisol. Conjugated metabolites of cortisol and other steroids are not found.

The finding of high plasma cortisol concentrations in pregnancy is well established, but the question of total adrenal cortical secretion in pregnancy is not clear. Cope & Black (36) used cortisol-4-<sup>14</sup>C as a tracer and found that the production of cortisol in normal pregnancy ranged from 20 to 40 mg. per day compared with 5 to 23 mg. per day in nonpregnant females, using the same technique. The excretion of 17-ketogenic steroids also is increased in

normal pregnancy. They also cite the work of Venning in 1946. She found increase in free cortical hormone (as determined by bioassay) in normal pregnancy.

A detailed study of pyrogenic steroids in man was reported by Kappas *et al.* (109). They found that the pyrogenic action of etiocholanolone was changed by removing the hydrogen from the hydroxyl group on C-3, and was completely eliminated by conjugation. The cortisol derivatives 11 $\beta$ -hydroxy-etiocholanolone, pregnanolone, and pregnanediol were pyrogenic, while progesterone had slight fever-producing capacity. 21-Hydroxypregnanedione was pyrogenic in occasional persons, but 17 $\alpha$ -pregnanolone and tetrahydrocortisone were not. The materials have to be injected intravenously over at least a 30 min. period. The pyrogenic steroids are of importance in certain patients with periodic fever and may have more important general significance.

Some work by Kvam & Parks (119) emphasizes the relationship of carbohydrate and protein metabolism very well. Glucose-6-phosphatase and fructose diphosphatase activity of livers of adrenalectomized rats was greatly increased by cortisol, but the increase was inhibited by the methionine antagonist, ethionine; the inhibition was reversed by methionine. These increases in enzyme activity represent an adaptive process involved in synthesis of new protein. In the presence of ethionine, both sugar and glycogen levels of the liver rose after cortisol but much less than those found in animals where the adaptive enzyme formation can occur. The enzyme changes did not occur in adrenalectomized rats on high sucrose feeding, but occurred in normal rats on sucrose feeding. Hess *et al.* (93) found that liver phosphorylase and glucose-6-phosphatase activities were decreased in fasted adrenalectomized rats but were increased above control levels by cortisone administration.

Mills & Thomas (150) found that inorganic phosphorus concentrations of plasma and whole blood fall after intravenous injection of cortisol. The arteriovenous difference widened when the arterial level was falling. The uptake in muscle was roughly equivalent to that which disappeared from blood. Liver vein blood was similar to arterial blood. Aldosterone and deoxycorticosterone did not have this effect. The rise in glycogen content of the liver was the first and most rapid effect.

The role of glucocorticoids in carbohydrate metabolism was studied in the response of mice to bacterial endotoxin by Berry *et al.* (15, 16). They found that the total carcass carbohydrate of poisoned mice was very low, whereas the carcasses of rats protected by cortisone had three to four times as much carbohydrate as the control mice. When given a dose of endotoxin which is lethal even for cortisone-treated mice, the carcass carbohydrate still was two to three times that of mice not treated with cortisone. Endotoxin prevented conversion of injected glucose to liver glycogen but not to muscle glycogen. In the presence of endotoxin, mouse liver mitochondria release adenosinetriphosphate at a rate like that released by dinitrophenol. Nitrogen

balance studies indicated that the mice treated with cortisone increased nitrogen excretion above that of controls and mice treated with endotoxin, and that the increased nitrogen mobilization was roughly parallel to increased carbohydrate stored in the cortisone-treated animals. Fukuda & Matsumoto (69) found that leukocytosis following initial leukopenia was a regular phenomenon in rabbits given typhoid-paratyphoid vaccine but was abolished by adrenalectomy. Cortisone restored the leukocytotic response. After tolerance to vaccine occurred, the leukocytotic response was diminished, but this was restored by pre-treatment with cortisone.

The interaction of insulin, corticoids, and growth hormone on glucose metabolism has been studied by Morgan *et al.* (154). Transport of glucose across the cell membrane of isolated perfused rat heart was a rate-limiting step in normal and diabetic hearts. The rate was decreased in diabetic hearts and was not accelerated by hypophysectomy or adrenalectomy. Anoxia stimulated transport, but insulin stimulated transport much more than anoxia. Phosphorylation capacity was greatly increased by anoxia. Phosphorylation was depressed in diabetic muscle, but hypophysectomy abolished the depression, and adrenalectomy partially relieved the depression of phosphorylation. Growth hormone or cortisol restored the depression of phosphorylation in adrenalectomized or hypophysectomized rats. These findings justify the conclusions that insulin and the level of aerobic metabolism regulate glucose transport and that phosphorylation is regulated by growth hormone and cortisol and the presence or absence of aerobic metabolism. Marshall & Engel (138) gave growth hormone, in a dose which regularly leads to hyperglycemia and glycosuria in normal tube-fed rats, to deoxycorticosterone acetate- or saline-maintained adrenalectomized rats and found no hyperglycemic effect. Cortisol restored the hyperglycemia response. Growth hormone was incapable of inhibiting glucose uptake by isolated diaphragm of adrenalectomized rats unless they were pre-treated with cortisone.

Manchester, Randle & Young (135) studied glucose uptake and incorporation of amino acids of isolated rat diaphragm in response to cortisol, insulin, and growth hormone. Both hypophysectomy and adrenalectomy raised glucose uptake while treatment of intact or hypophysectomized rats with growth hormone or adrenalectomized rats with cortisol depressed it. Adrenalectomy and hypophysectomy did not influence uptake of glucose induced by 200 mU. of insulin per ml. of medium, but both enhanced the uptake when 0.1 to 1.0 mU. of insulin per ml. of medium were used. Hypophysectomy depressed and growth hormone increased the incorporation of glycine into protein, but neither altered the magnitude of stimulation of incorporation by 1.0 mU. of insulin per ml. of medium. Adrenalectomy raised and cortisol, administered to intact or adrenalectomized rats, depressed incorporation of glycine into isolated diaphragm. Adrenalectomy increased but cortisol diminished the effect of 1.0 mU. insulin per ml. of medium. They concluded that growth hormone directs insulin towards stimulation of pro-

tein synthesis in part by restraining the action of insulin on carbohydrate metabolism.

Insulin enhances the uptake of glucose by rat epididymal fat tissue. By injecting cortisone for three days into rats, Correa *et al.* (40) were able to suppress completely the uptake of glucose induced by doses of insulin to which the fat tissue is sensitive normally.

Hausberger & Ramsay (87) examined the islets of Langerhans in mice bearing spontaneous ACTH-producing tumors. The islets showed hypertrophy, hyperplasia, and degranulation of beta cells. Islet hypertrophy was less marked in animals on a restricted diet. The mice also had marked hypertrophy of the adrenal cortex, atrophy of lymphoid tissue, polyuria, and sometimes hyperglycemia. Corticosterone or 11-dehydrocorticosterone pellets implanted into mice caused comparable obesity and islet changes.

Hollifield & Parson (100) implanted pellets of 11-dehydrocorticosterone subcutaneously in young DBA mice. Increase of fat occurred in spite of weight loss, decreased food intake, and decreased running activity. Adipose tissue of mice treated with pellets had greater lipid synthesis from  $^{14}\text{C}$ -labeled acetate than that of cholesterol-treated controls. Hollifield & Parson postulated that increased gluconeogenesis caused increased insulin production and, therefore, increased fat synthesis. An alternate explanation is suggested by Gordon's work (77). Corticosterone ingested by normal persons regularly suppressed mobilization of fat as measured by non-esterified fatty acid response compared with fasting, whereas cortisone increased this response. Some very obese people had no rise in non-esterified fat level in response to fasting. It is possible that corticosterone or 11-dehydrocorticosterone may be important factors in some instances of obesity.

Blecher & White (18) found that cortisol inhibited anaerobic glycolysis in cell-free preparations of rat lymphosarcoma when glucose was substrate but not when hexose monophosphate or fructose diphosphate were substrates, but deoxycorticosterone inhibited anaerobic glycolysis when any of above substrates were used. Apparently DOC stimulated hydrolysis of labile phosphate esters. Deoxycorticosterone and cortisol did not influence hexokinase activity of extracts of muscle or lymphosarcoma. Complete negation of the inhibition was obtained by adding yeast hexokinase and an exogenous adenosinetriphosphate generating system. Thus cortisol and DOC appeared to exert their effect on the availability of adenosinetriphosphate.

Lacroix & Leusen (122) found that utilization of glucose and pyruvate by surviving heart slices was markedly impaired by cortisone acetate administered to rats for 14 to 16 days before sacrifice. Alpha ketoglutarate and succinate stimulated oxygen consumption of slices to the same extent in the cortisone group as in the control. These experiments suggest a block between pyruvate and alpha ketoglutarate.

Kowalewski (116) showed that cortisone inhibited the growth of Nobles' transplanted osteosarcoma in rats and more particularly it inhibited uptake



of  $^{35}\text{S}$  by sulfated mucopolysaccharides of the lesions. Cortisone acted as an inhibitor of synthesis of certain mucopolysaccharides of connective tissue. McCluskey & Thomas (131) treated rabbits with papain in order to soften cartilage. Prednisone, cortisone, or cortisol prevented restoration of normal cartilage rigidity and accumulation of basophilic substance in cartilage matrix. Uptake of  $^{35}\text{S}$  was impaired by prednisone. Wright *et al.* (232) determined the hexosamine to collagen ratio of skin before and after treatment with steroids and found that the ratio decreased 5 per cent or more in 32 subjects with a mean decrease of  $12.9 \pm 1.5$  per cent. These experiments point to the fact that glucocorticoids interfere with the synthesis of chondroitin sulfate in cartilage and skin and of other mucopolysaccharides in ground substance. This evidence is extremely important in relation to some of the complications of corticoid therapy.

Estrogens, on the other hand, increased the amount of mucopolysaccharides in skin of immature monkeys (223). The estrogen effect was not influenced by castration in males or by cortisone. Edgren & Calhoun (56) noted that the uterine growth of intact immature mice was stimulated by injection of  $0.3 \mu\text{g}$ . of estrone. Cortisone and cortisol had no inhibitory effect. Deoxycorticosterone and aldosterone had a definite inhibitory effect equivalent to one-tenth that of progesterone.

Spaziani & Szego (205) used radioactive  $^{24}\text{Na}$  to determine the effect of estradiol and cortisone on immature rat uterus. Estradiol caused a distinct uptake of  $^{24}\text{Na}$  and water by the uterus with the maximum effect at  $2\frac{1}{2}$  hr. There was no effect on other tissues. Cortisol blocked the increase in uterine water and  $^{24}\text{Na}$ .

The effects of steroids on protein synthesis and metabolism have been studied directly with labeled amino acids by Wool and co-workers (229 to 231). Starvation increased the incorporation of several  $^{14}\text{C}$  amino acids into proteins of isolated rat diaphragm, and a high carbohydrate diet enhanced this effect. This adaptive change occurred in spite of absence of the pituitary. The incorporation of  $^{14}\text{C}$  from glucose, carboxylic acids, and bicarbonate into proteins of diaphragm was enhanced by adrenalectomy and decreased by cortisone. Incorporation of pyruvate-3- $^{14}\text{C}$  was not influenced by twenty-fold change in pyruvate concentration. Incorporation of  $^{14}\text{C}$  from glucose-1- $^{14}\text{C}$  and glucose-6- $^{14}\text{C}$  was of the same magnitude, which means that glucose must be metabolized via Embden-Meyerhof pathway. Cortisone in huge doses produced marked reduction of incorporation of amino acids into rat diaphragm of both intact and adrenalectomized rats. Deoxycorticosterone was without effect. Adrenalectomy increased incorporation of amino acids. A hundred-fold change in concentration of phenylalanine was without effect. These results are direct corroboration of the anti-anabolic effects of glucocorticoids.

Aschkenasy & Wellers (2) fed a protein-free diet to rats with total adrenalectomy. Synthesis of liver glutathione was prevented even if these rats were given cystine or glutamic acid alone but did occur when both were fed or cortisone was given. Glucocorticoids are necessary for mobilization of cystine

and glutamic acid from tissue stores. Gaebler *et al.* (70) contrasted the incorporation of glycine, alanine, and ammonium citrate into protein under influence of growth hormone with the catabolic effect of ACTH. The anabolic nature of growth hormone was supported. The rise in glycine and alanine in disproportionate amounts after administration of ACTH supported vigorous catabolic action of adrenal corticosteroids. It appears, therefore, that glucocorticoids have a distinct primary catabolic action as well as an antianabolic action both *in vivo* and in isolated tissues. Sobel *et al.* (203) cite experimental evidence for selective or disproportionate loss of nitrogenous substances from heart and kidneys following starvation or starvation and cortisone. Therefore, equivalent nitrogen loss induced by several conditions does not necessarily cause the same changes among individual proteins. In other words, all forms of cachexia are not equivalent.

Grossfeld (80) found high concentrations of cortisol inhibited growth and depressed respiration and production of mucopolysaccharide in tissue culture. This probably is significant in wound healing impaired because of excessive cortisol. Prednisolone was three times as effective as cortisol. Cortisol excess stimulated anaerobic metabolism. Moscona & Karnofsky (155) studied effects of steroids on chicken embryos. A single dose of cortisone applied to the allantoic vesicle or chorioallantoic membrane of the chick embryo from the third to eighth day caused a variety of malformations including weight inhibition, defects in facial bones and tibia, and exteriorization of viscera. In later embryos facial bony defects were not found, but inhibition of weight and feather growth were marked. This has obvious implications in teratogenesis.

Reis (172) attempted to develop a means of assay of the synergistic effect of steroids and adrenergic compounds. Cortisol and cortisone were found to increase the sensitivity of bulbar conjunctival vessels to topical norepinephrine. 11-Deoxycortisol and 11-dehydrocorticosterone were without effect but DOC was strikingly effective in some patients. Haydu & Wolfson (89) found that simultaneous administration of cortisone potentiated the effect of heat and cold on DPN toxicity. Injections of DPN in mice produced toxic effects similar to those produced by nicotinamide analogues, and heat and cold potentiated the toxicity. Chappel *et al.* (33) administered mineralocorticoids, deoxycorticosterone acetate, and 9 $\alpha$ -fluorocortisol to rats treated also with isoproterenol. There was an increase in mortality and in severity of heart lesions. Cortisone or triamcinalone pretreatment had no appreciable effect. The infarct-like heart lesions were more severe and uniform after subcutaneous administration of isoproterenol than those produced by epinephrine or norepinephrine.

Williams & Angerer (227) found that adrenal cortical extract increased the potential difference across frog skin, while decreased levels of adrenal cortical hormones produced significantly reduced potential differences across the frog skin.

The performance of work, measured as left ventricular work index, in

the rat heart-lung preparation was studied by Sayers & Solomon (190). Cortisol, corticosterone, and aldosterone increased the left ventricular work index, but 11-deoxycortisol and tetrahydrocortisol had no effect. Tetrahydrocortisol was inhibitory at high levels, and so was corticosterone. This work is important not only as a measure of physiological effects and significance of dose level for a given effect of various steroids, but also as a model for many physiological studies.

#### BIOGENESIS AND CONTROL OF ALDOSTERONE SECRETION

The biogenesis of aldosterone will be considered separately since there are a number of unique conditions involved. Farrell (62, 63) produced a crude acetone-soluble fraction of beef pineal tissue which was assayed for steroidogenic activity in the decerebrate dog. Aldosterone secretion was markedly stimulated without a significant effect on cortisol output. He also found a substance in the pineal which inhibits the output of both aldosterone and cortisol. The role of the anterior pituitary in control of aldosterone secretion in experimental secondary hyperaldosteronism was studied by Davis *et al.* (46). Cortisone in a dose of 100 to 200 mg. caused a decrease in aldosterone and corticosterone output. Hypophysectomy caused further decline in aldosterone and corticosterone output. In hypophysectomized dogs,  $\alpha$ - and  $\beta$ -melanocyte-stimulating hormone had no effect on aldosterone and corticosterone excretion. Hypophysectomized dogs did not respond to low salt intake with increased aldosterone in adrenal vein blood, and in three such dogs aldosterone excretion was not increased by acute thoracic inferior vena caval constriction. Aldosterone secretion responded to ACTH given to hypophysectomized dogs on a low salt diet. Therefore, the anterior pituitary plays an important role in increased production of aldosterone in secondary hyperaldosteronism, probably by keeping steroidogenesis at a high level.

Davis (45) produced hypothalamic lesions in dogs with inferior vena cava constriction with the following results. Striking sodium retention and aldosterone secretion continued. Destruction of every area of the hypothalamus was accomplished in at least one animal. In seven animals, the aldosterone output remained higher than normal, but in those which survived, a progressive decline in aldosterone secretion occurred. In the three with decline, there was bilateral injury of the median eminence and adrenal atrophy of the zona fasciculata and reticularis. Chronic hypothalamic lesions were without effect unless the median eminence was injured. These findings support the conclusion that steroidogenesis at a high level favors aldosterone secretion too. Cross-circulation between normal dogs and those on which thoracic inferior vena caval constriction experiments were performed by Yankopoulos *et al.* (234) resulted in an average fivefold increase of aldosterone secretion by the normal adrenal gland. Bahn *et al.* (7) noted atrophy of both the zona glomerulosa and inner adrenal cortical zones six weeks after complete anterior and posterior hypophysectomy and subtotal removal of the pars tuberalis in the dog. Reimplantation of a portion of the pars distalis

resulted in partial maintenance of the mass of each adrenal cortical zone.

An interesting experiment of nature was reported by Siebenmann (197). A patient with Cushing's syndrome underwent exploration of the left adrenal. Severe fatal pancreatitis ensued, and, at autopsy, the left adrenal had an intact zona glomerulosa but the rest of the cortex was necrotic. Ninety-five per cent of the steroid isolated from this adrenal was aldosterone. Only 3.6 per cent of the total steroid isolated from the hyperplastic right adrenal was aldosterone, whereas cortisol accounted for 81 per cent, cortisone for 2.4 per cent, and corticosterone for 12.8 per cent.

Ayres *et al.* (6) used bovine adrenal capsule strippings to study steroidogenesis *in vitro* since aldosterone is produced preferentially by this preparation. Labeled progesterone, DOC, and corticosterone were all converted to aldosterone. At least 50 per cent of the aldosterone was derived from corticosterone, and with high concentrations as much as 92 per cent was derived from corticosterone. Progesterone was a major precursor for both aldosterone and corticosterone. The major sequence appears to be progesterone to DOC to corticosterone to aldosterone. Cholesterol can yield corticosterone presumably through progesterone. Mulrow & Cohn (156) found that human adrenal slices will synthesize aldosterone from corticosterone 4-<sup>14</sup>C.

Physiological factors which control aldosterone secretion continue to occupy the attention of many investigators. Bartter & Gann (11) reviewed factors which influence the secretion of aldosterone and particularly the hemodynamic factors. They cited evidence which supports the theory that variation of pulse pressure in the carotid arteries results in control of secretion: decrease in pulse pressure acts as a stimulus to secretion and increase of pulse pressure acts as a depressant. Cox *et al.* (41) produced a sodium and water depletion in normal subjects. Aldosterone excretion was increased in all. The cell water was decreased as much as the extracellular water. Romani *et al.* (176) measured aldosterone excretion in normal ambulatory subjects compared with that of normal recumbent subjects. The ambulatory subjects excreted an average of 8.5  $\mu$ g. per day compared with an average of 4.6  $\mu$ g. per day for recumbent subjects. Assali *et al.* (3) found that quiet standing produced marked decrease in water and sodium chloride excretion, and in glomerular filtration. Venous congestion of the lower extremities produced renal and systemic effects similar to those of quiet standing. Alcohol did not inhibit antidiuresis. Aldosterone excretion did not show diurnal variation but rose markedly during standing. There is a decrease in glomerular filtration rate which probably accounts for the antidiuresis in pregnancy. Patients with diabetes insipidus have normal aldosterone metabolism with normal response to sodium depletion and water deprivation, according to the observations of Küchel *et al.* (118). Olsen *et al.* (163) found that aldosterone excretion is greater than normal in obese subjects.

One previously adrenalectomized patient was found to excrete measurable amounts of aldosterone during pregnancy, but, after delivery, this patient excreted just measurable amounts of aldosterone. Some adrenal regeneration cannot be excluded however (217).

Denton *et al.* (49, 50) studied factors which induce aldosterone secretion by measuring the salivary sodium to potassium ratio in sheep. The parotid duct was cannulated. Sheep produce from 4 to 8 l. of saliva a day, and as a consequence, can be depleted of sodium by this means. Cortisol had no effect on the ratio. Rapid infusion of 240 m.eq. of a sodium salt into a sodium-depleted sheep caused a change in the cation ratio in 120 to 160 min. Anesthesia or excitement abolished this rapid correction. One adrenal was transplanted to the neck for convenient blood sampling in order to check the salivary gland responses by aldosterone determinations. Lowering sodium and increasing potassium in the adrenal artery did not change parotid secretion until many hours later. Segmental central nervous system removal revealed that decerebration had an effect like sodium repletion, i.e., cessation of aldosterone secretion. Removal of the brain rostral to the pons interfered with changes following rapid sodium repletion. When the level of decerebration was at the quadrigemina and portion of the mammillary body, the response to sodium depletion and repletion was normal. The ingenious experiments of Denton *et al.* extended to studying the appetite of sheep for various saline solutions after sodium depletion; sheep had remarkably accurate appetites for sodium. The measurement by these workers of parotid secretion and transplantation of adrenal glands to the neck are very useful tools.

McDonald & Reich (132) also transplanted the adrenal of sheep to the neck vessels with the following results. In the resting state, cortisol was produced predominantly; in moderate sodium depletion states, aldosterone was always present without an increase in cortisol or corticosterone; in sodium-depleted animals, ACTH caused a greater than normal increase in cortisol and corticosterone excretion but also increased aldosterone secretion. In normal subjects Crabbé *et al.* (43) were able to show that ACTH caused an increased secretion of aldosterone. After short-term stimulus there was a three-to fourfold increase, but in chronic stimulus of 12 days excretion almost doubled.

#### METABOLIC EFFECTS OF ALDOSTERONE AND RELATED STEROIDS

That patients with primary hyperaldosteronism seldom are edematous has posed an important question about the role of aldosterone in edema formation in so-called secondary hyperaldosteronism. August & Nelson (4) gave large doses of aldosterone or DOCA to five patients with Addison's disease. There was an escape from the sodium-retaining property after a few days of sodium retention. Changing the dosage of glucocorticoids did not influence sodium retention, nor did Pitressin. Strauss (208) examined this same problem in normal men using large doses of 9 $\alpha$ -fluorohydrocortisone. The sodium content of the body increased about 200 m.eq. and then failed to increase further. Reubi (173) administered 130  $\mu$ g. of aldosterone per day to four edematous patients. In all, sodium retention was enhanced, but after cessation, diuresis occurred in one. None of the four patients had excessively high initial aldosterone excretion. It is clear that a plateau of sodium retention must occur, because, otherwise, excessive edema would be the rule

whenever primary aldosteronism or excessive and prolonged exogenous DOCA administration were present. A mechanism of sodium excretion in spite of mineralocorticoid excess has not been elucidated, but it has been known for a long time that dogs also develop an escape from the sodium retention produced by large doses of DOCA (171). It is quite significant that the dogs developed polydipsia and polyuria. Patients with primary aldosteronism have polyuria and polydipsia, and this may well be the mechanism which results in sodium excretion. Gordon & Eichenholz (78) found that profound water diuresis in a patient with primary aldosteronism resulted in marked decrease in tubular reabsorption of sodium without changing glomerular filtration significantly. This evidence suggests the importance of water excretion in maintaining sodium equilibrium when there is an excessive amount of mineralocorticoid present.

Mills *et al.* (151) were able to differentiate the effects of cortisol, aldosterone, and deoxycorticosterone on the electrolyte excretion of healthy males. Doses of the three steroids were adjusted so the sodium retention was the same order of magnitude. In the afternoon, cortisol increased potassium output by an amount equivalent to sodium retention and thus hardly changed the hydrogen ion excretion. Thus, the hydrogen ion to potassium ratio was greatly reduced. Aldosterone and DOC increased excretion of both potassium and hydrogen ion so the ratio remained unchanged. At bedtime, cortisol caused a rise of potassium excretion much greater than sodium retention balanced by a fall of hydrogen ion excretion. Aldosterone and DOC increased potassium and hydrogen ion excretion as in the afternoon but did not change the ratio of the two. When acetazolamide was given to stop hydrogen ion excretion, all three steroids decreased sodium and increased potassium excretion. Morning experiments were subject to too much random variation to be interpreted. These experiments illustrate beautifully the similarity of action of aldosterone and of DOC and the importance of paying attention to the diurnal or circadian variations in physiological functions and particularly adrenal function and electrolyte metabolism (53, 81, 83, 84).

Doe *et al.* (53) studied the variation of sodium, potassium, creatinine, 17-hydroxycorticosteroids, and magnesium every 3 hr. throughout a 30 hr. period in normals and patients with adrenal disorders. All subjects received an identical feeding every 3 hr. The peak excretion of sodium, potassium, and 17-hydroxycorticosteroids occurred in the morning hours from 6 to 9 and 9 to 12. Graded doses of cortisol were given to patients with Addison's disease in such a way that the peak cortisol levels would occur at a time significantly displaced from the usual one. A definite increase in both sodium and potassium excretion occurred at the peak of corticoid excretion regardless of the time of day. However, 5.0 mg. cortisol hemisuccinate given intravenously every 3 hr. did not completely obliterate the peak excretion of sodium and potassium during the day.

Hills *et al.* (96) studied the excretion of sodium chloride in relation to magnesium and other ions in human subjects. Augmented sodium chloride



excretion resulted in increased urinary magnesium excretion, and decreased sodium chloride excretion was accompanied by decreased magnesium excretion. Cortisol administered intravenously caused distinct sodium chloride retention in a patient with adrenal insufficiency. In a study of magnesium excretion every 3 hr. through the 30 hr., Doe *et al.* (53) found that in normal subjects as well as patients with adrenal cortical disorders, the magnesium excretion was highest at night in all groups at a time when sodium excretion was usually the lowest. This suggests that sodium and magnesium excretion do not follow the same pattern. Miller *et al.* (148) found that magnesium excretion is not altered by stimulation of endogenous aldosterone production and did not follow the sodium excretion. Brandt & Glaser (23) could demonstrate no effect of cortisol or adrenalectomy on the disappearance rate of  $^{25}\text{Mg}$  from the blood of rats.

Knowlton (113) compared the effect of DOCA and cortisone acetate on the rat skeletal muscle electrolytes of adrenalectomized rats. Cortisone produced severe hypertension without causing any significant changes in muscle sodium or potassium. On the other hand, DOCA given in amounts to produce some degree of hypertension produced an expected increase in sodium and decrease of potassium concentration in muscle. The cortisone hypertension occurred regardless of sodium intake, but the hypertension and electrolyte effects of DOCA were dependent on a high sodium intake. Krück (117) studied the extrarenal electrolyte shifts in rats six hours after nephrectomy and adrenalectomy and administration of either 0.5  $\mu\text{g}$ . of aldosterone or 15  $\mu\text{g}$ . DOCA. Aldosterone caused an increase of the intracellular sodium to potassium ratio; DOCA caused a smaller decrease in muscle potassium but no change in sodium; aldosterone caused a distinct rise in plasma  $\text{CO}_2$ .

It is interesting that the insertion of a fluorine atom at the 9 $\alpha$  position of deoxycorticosterone increased the potency of its sodium retaining and potassium excreting qualities about 12 times over that of the parent compound as measured by Kagawa & Jacobs (108).

Vander, Wilde & Malvin (215) determined the effect of various steroids on the excretion of sodium by the kidney using stop-flow technique. The distal tubular segment can lower the sodium concentration to zero virtually independent of plasma sodium concentration. In adrenalectomized animals, the minimal distal tubule concentration of sodium was not as low as normal, and the minimal sodium concentration in the distal tubule increased also in direct proportion to serum concentration. Adrenalectomy, therefore, reduces the maximal concentration gradient across the tubular membrane. Cortisone does not repair the defect, but aldosterone restores the ability of the distal tubule to lower sodium concentration even when plasma sodium is elevated. Aldosterone does not alter proximal tubular sodium reabsorption. A spiro-lactone, 3(3-oxo-17 $\beta$ -hydroxy-19-nor-4-androsten-17 $\alpha$ -yl-1) propionic acid gamma lactone (SC 8109), blocks the renal effects of aldosterone and DOC. Wilbrandt (226) made the intriguing suggestion that ion transport in the



kidneys, and in other tissues too, occurs by means of lipoidal chelation of the ions.

Singer (198) used the same aldosterone inhibitor, SC 8109, and measured the aldosterone content of the adrenal vein blood of rats and found no effect after a week of administration. When SC 8109 was combined with a low salt diet, though, the aldosterone production increased dramatically above that on the diet alone.

DasGupta & Giroud (44) used a spiro lactone in rats with aminonucleoside nephrosis and produced loss of ascites and edema without influencing secretion of aldosterone. Many therapeutic trials with various spiro lactone aldosterone antagonists have been reported. In some refractory edema states, especially when associated with high aldosterone secretion, spectacular results have been obtained. Cirrhosis of the liver is a disease in which sodium excretion often approaches zero and the spiro lactones have been particularly helpful. It is common experience that combining a spiro lactone with a benzothiadiazine compound often greatly enhances sodium diuresis and decreases the potassium loss induced by the latter (31, 32, 58, 200).

A recent fascinating finding by Cejka *et al.* (32) and Schlattmann *et al.* (194) that heparin and heparinoids have a natriuretic effect may have profound effect on understanding of how steroids act. It has been found that aldosterone excretion decreased during administration of the heparinoid to patients with secondary hyperaldosteronism.

The metabolism of aldosterone was studied by Hunter & Hunter (105) using tritium-labeled aldosterone in six cirrhosis patients. All had increased aldosterone excretion in the urine. There was a decreased glucuronide formation in four of the six and a greater fraction was extractable at pH 1.0. Aldosterone disappearance as measured by half life was prolonged in cirrhosis (167).

Ross (181) administered aldosterone and corticosterone simultaneously and separately to a normal subject. Potassium diuresis was greater with both than with aldosterone alone and occurred both day and night. Aldosterone alone and aldosterone plus cortisol were given to two patients with Addison's disease with essentially the same results in that potassium excretion was enhanced by more than 40 per cent by the combined therapy. There was no change in creatinine clearance. It is suggested that the regular occurrence of potassium wastage in primary hyperaldosteronism is related to slightly excessive secretion of corticosteroids compared with instances of secondary hyperaldosteronism.

An unusual patient is reported by Hökfelt *et al.* (99). A 55-year-old woman had periodic edema and excreted excessive amounts of cortisol metabolites equivalent to 200 to 300 mg. of cortisol per day during periods of edema. Aldosterone excretion was below normal during this time. Pregnanetriol-11-one and considerable amounts of cortolone were isolated from the urine. An interesting and perhaps related phenomenon was reported by Dudley *et al.* (54). A patient with Addison's disease was given a constant dose

of cortisone during and after thoracoplasty. Obviously, there was no aldosterone secretion and yet there was sodium retention during and immediately after the procedure. This simply means that many factors condition the organism in its over-all sodium and potassium balance.

A detailed review of primary aldosteronism will not be reported, but a few aspects will be considered. Gornall *et al.* (79) administered 0.4 to 0.5  $\mu\text{g.}$  of aldosterone per 100 gm. body weight three to six days a week to young rats. Hypertension as measured by systolic blood pressure was produced over a period of three to six months. Equivalent doses of 9 $\alpha$ -fluorohydrocortisone and 2-methyl-9 $\alpha$ -fluorohydrocortisone produced similar results. Reserpine was not protective; in fact, it was toxic and led to a rise in blood pressure. Hypertension can result in three to eight weeks from administering hypertonic sodium chloride solution to adrenalectomized rats (68).

The renal effects of primary hyperaldosteronism are reviewed by Barrett *et al.* (10). Their patient had hypokalemia without alkalosis and a labile blood pressure. Romani *et al.* (177) were unable to increase aldosterone excretion by use of ACTH in a patient with an adenoma. Zimmerman *et al.* (235) reported five patients including one suffering from carcinoma with metastases and one with hyperplasia. In the patient with hyperplasia but not the one with carcinoma, it was possible to increase aldosterone excretion with ACTH. Baulieu *et al.* (12) reported a patient in whom aldosterone excretion was decreased by 9 $\alpha$ F- $\Delta^1$ -cortisol and was increased by posterior pituitary extract and by ACTH. They were unable to find evidence of secretion of corticosterone. Not all patients with hypertension and associated hypokalemic alkalosis have primary aldosteronism.

Molnar *et al.* (153) described a patient with isolated aldosterone deficiency. Given a dose of 200 m $\mu$  per day of aldosterone orally, he excreted 11 and 18  $\mu\text{g.}$  per day in the urine, and given 300  $\mu\text{g.}$  per day he excreted 18  $\mu\text{g.}$  per day. This corresponds to estimates of the fraction of secreted aldosterone which is identified in the urine normally. Hill *et al.* (95) also report a patient with isolated aldosterone deficiency.

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## REPRODUCTION<sup>1,2</sup>

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### INTRODUCTION

The review period of mid-1959 to mid-1960 has witnessed the publication of over 5000 titles in reproductive anatomy, physiology, biochemistry, histochemistry, and the several clinical manifestations of reproductive processes. Thus it is possible to review completely but a small segment of these interesting papers. Specific and detailed attention will be given to the basic concepts of reproductive mechanisms, and much less attention will be given to the clinical aspects.

Since the last review published in this series, informative and useful monographs on several aspects of reproductive endocrinology have appeared, including those edited by Cole & Cupps (1, 2), Eckstein (3), Gorbman (4), Lloyd (5), and Villee (6). Also appearing in book form are the proceedings of conferences on modern trends in obstetrics and gynecology (7), trophoblasts and its tumors (8), and the vagina (9), and of the Laurentian Hormone Conference (10).

### NEUROENDOCRINE FUNCTION, HYPOPHYSIS, AND GONADOTROPINS

*Neuroendocrine function.*—The recent literature abounds with reports indicating that neuroendocrinology is gradually becoming established on a firm foundation. Sawyer and his associates (11 to 15) have been concerned with a number of physiological studies on some interactions between the brain and the pituitary-gonad axis in the rabbit. Priming the estrous rabbit with moderate doses of estrogen for two days or the anestrus rabbit for a longer period induced the release of pituitary ovulating hormone in response to artificial stimulation of the vagina in 40 to 45 per cent of animals. This response was blocked in estrous estrogen-treated rabbits by pentobarbital, atropine, dibenamine, and reserpine. Sawyer & Markee (11) emphasized the facilitatory function of estrogen in reflexogenous ovulation. They related estrogen action to two centers in the hypothalamus of the rabbit: (a) one located in the basal tuberal region (which responds to electrical stimulation

<sup>1</sup> The period surveyed included June 1, 1959, to May 31, 1960, but the April and May issues of some of the journals printed in and outside of the United States of America were not available in time to be considered.

<sup>2</sup> The following abbreviations have been employed in this chapter: ACTH (adrenocorticotropin); DPN (diphosphopyridine nucleotide); FSH (follicle-stimulating hormone); HCG (human chorionic gonadotropin); HPG (human urinary pituitary gonadotropin); ICSH (interstitial cell-stimulating hormone); LH (luteinizing hormone); LTH (luteotropic hormone); PAS (periodic acid Schiff); PMS (pregnant mare serum gonadotropin); TSH (thyrotropin).

by inducing ovulation in the estrogen-treated rabbit, but in which discrete lesions block copulation-induced ovulation); and (b) the other located in the mammillary region (which appears to control mating behavior).

Electroencephalographic (EEG) changes have been recorded from the brain of the conscious unrestrained female rabbit after coitus and vaginal stimulation. In the estrous estrogen-primed rabbit with electrodes permanently implanted in cortical and deep regions of the brain, the following sequence of EEG changes was observed: (a) a phase of sleep spindles lasting from several seconds to 30 minutes or more, and (b) a phase of "hippocampal hyperactivity" characterized by an eight per second high amplitude synchronous EEG record from the hippocampus and its projections. Sawyer & Kawakami (12) believe that these phases occur too late to be casually related to neurogenous activation of the release of pituitary hormone, but that their timing suggests they may themselves be induced by the "feedback" to the nervous system of pituitary hormones released in response to the coital or vaginal stimulus (12). The EEG after-reaction which ordinarily follows coitus in the female rabbit can be induced by "inhibitory-type" low-frequency (5/sec.), low-voltage (0.01 to 1.0 V) electrical stimulation applied through chronically implanted electrodes to the hypothalamus, the olfactory bulb, the septum, the hippocampus, or the amygdala. The EEG after-reaction occurred "spontaneously" after treatment with HCG, PMS, LH, LTH, and neurohypophyseal hormones, but the latency was greater than that following electrical stimulation. The effective pituitary hormones were those whose release mechanisms were activated by coital stimuli; therefore, the results are consistent with the hypothesis that the induction of the post-coital after-reaction may be mediated by pituitary hormones acting back on the nervous system. Kawakami & Sawyer (13) hypothesized that such a neuroendocrine negative feedback mechanism might serve to inhibit the release of superfluous ovulating hormone. Sawyer & Kawakami (14, 15), in their studies on the neuroendocrine correlates of changes in brain activity thresholds by sex steroids and pituitary hormones, ascertained the sex behavior as related to changes in thresholds of two opposing cerebral systems: (a) the EEG arousal threshold involving the brainstem reticular formation (b) and the EEG after-reaction threshold involving the rhinencephalon and hypothalamus. In the estrous or estrogen-primed female rabbit, injection of progesterone at first lowered both thresholds for a few hours and subsequently elevated them to supranormal levels until withdrawal or rebound brought them down again. They determined that the early phase is related to estrus and a lowered threshold of pituitary activation, whereas the elevated thresholds correlated with anestrus and pituitary inhibition. Testosterone, certain pituitary and placental hormones, seasonal influences, and prolonged treatment with female sex steroids affected the two thresholds differently. Sawyer & Kawakami concluded that changes in (a) are more closely related to sexual behavior, whereas alterations in (b) are correlated with pituitary thresholds for the release of ovulating hormone.

These five studies, all of a ground-breaking variety, lead us to the thesis that both the facilitatory and inhibitory effects of sex steroids on pituitary activation, as well as influences on behavior, are mediated by means of altered thresholds of cerebral activity. The ventromedian hypothalamic region of the hen could be either the actual site of progesterone "excitation" or merely a link in a neural complex mediating the effects of progesterone (16, 17). Destruction of this region apparently precludes the release of gonadotropic hormone that usually follows when progesterone is administered at appropriate times. Progesterone induced ovulation in the hen when injected into certain regions of either the hypothalamus or the caudal neostriatum but not in other parts of the forebrain (17). Neuroendocrine relationships have been the subject of recent reviews (18, 19).

Giving added credence to the link between neural and endocrine mechanisms is the work which shows that posterior pituitary polypeptides may play a role in the physiological regulation of gonadotropin release (20). Hypothalamic lesions in the supraopticohypophyseal tract of lactating rats produced a block of both milk ejection and milk secretion (21).

Coagulating lesions in the posterior portion of the midbrain and level of rostral pons and in the posterior diencephalon and rostral midbrain effected inhibition of ovulation in rats when concomitant mammillary peduncle damage was present (22). Ovulation was also blocked by mesencephalic lesions (23). Sawyer (24) summarized the status of our knowledge on the "nervous" control of ovulation. The facilitatory and inhibitory actions of ovarian steroids on ovulation may be attributable to the influence of these hormones on differential thresholds in the nervous system (24).

Female rats with bilateral lesions of the area from the mammillary body to the ventromedian nucleus displayed definite signs of delayed puberty, reproductive immaturity, viciousness, and imminent obesity (25). Anterior hypothalamic lesions in female rats were followed by precocious ovarian follicular growth with uterine and vaginal manifestations of estrogen secretion. Lesions damaging part of the arcuate nucleus have not only these sequelae, but are also followed by ovarian luteinization. Bogdanove & Schoen thus conclude that anterior lesions increase FSH release, whereas arcuate nucleus lesions increase both FSH and LH release (26). Dogs with destruction of the posterior median eminence showed depressed LH (ICSH) and FSH values (27). Destruction of the posterior median eminence (in 8 dogs) resulted in testicular and prostatic atrophy without evidence of adrenocortical or thyroid inhibition (28). Bilateral symmetrical electrolytic lesions which damage the arcuate nucleus and also the median eminence in the hypothalamus of adult female rats regularly produced atrophy of the reproductive tract and reduced thyroid function but were without consistent effect on adrenal morphology (29). Anterior pituitary tissue grafted into the anterior chamber of the eye of hypophysectomized rats lacking any connection with the median eminence did not retain any somatotrophic or gonadotropic activities but had some ACTH and thyrotropin activities (30).

Attempts were made to separate the pituitary gland from the hypothalamus in eight mature female rhesus monkeys by placing a piece of film between the cut ends of the pituitary stalk (31). One monkey died shortly after the operation. In two it was established that the stalk had not been divided, and normal menstrual cycles continued, as they did in a fourth, in spite of extensive damage to the stalk. In three of the monkeys, amenorrhea set in despite variable degrees of regeneration of hypothalamohypophyseal vascular connections. One animal underwent two phases of uterine bleeding in spite of complete structural and vascular separation of the pituitary gland from the hypothalamus. The neural process was found to be atrophic in the seven animals, and all developed diabetes insipidus. The severity of polyuria could be correlated with the amount of damage to the median eminence rather than to the stalk (31).

*The pituitary.*—Somatotropin, corticotropin, and gonadotropin have been detected in serial cultivation of cells obtained from human pituitary. Two of the cell lines produce FSH and ICSH (LH) when in suspension culture, and one, S 4374-M, has been frozen and recultivated in both stationary and suspension types of culture with media containing 10 per cent of human, equine, or bovine serum, with resumption of production of hormone. The gonadotropin-producing cells have the microscopical appearance of a chromophobe type of pituitary cells and stain characteristically with iron-PAS (32).

With the use of differential centrifugation techniques on pig pituitaries, TSH was found in mitochondria, LH and STH in acidophilic granules, and FSH was distributed over several fractions. ACTH was found in the microsomes and in the supernatant fluid, depending upon method of assay employed (33).

It has been found possible to irradiate the monkey pituitary with single doses of 4500 and 9000 rep deuterons without serious injury to nerves or to those parts of the brain lying in the path of the beam. Vascular trunks very near the pituitary were not severely damaged; no rupture of these vessels occurred and they were not occluded by thrombi. Deuteron irradiation reduces the size of the pituitary of such monkeys. The higher dose caused disorganization of lobular architecture with profound destruction of parenchyma and replacement by fibrous tissue. A few normal-appearing chromophils could be found in the anterior lobes of all pituitaries (34).

Since the ferret is used quite frequently in reproductive studies, it seems germane to include a report on the histology and cytology of the pituitary of this species. Six distinct types of cells can be distinguished in the pars distalis of the pituitary gland of the female ferret after staining by PAS-orange G. The first of these types contains coarsely granular PAS+ material; the second and third, granules which stain with Alcian blue, with or without PAS+ material; the fourth, apparently equivalent to the "carminophil" of other species, stains deep orange in response to a combination of staining with Orange G and a weakly PAS+ reaction. The fifth is ordinary

acidophil, and the sixth, the chromophobe. The pars tuberalis is extensive and may be compact or lobulated; the pars intermedia contains two types of cells but is seemingly without secretory characteristics. The neural process consists of a central zone, which resembles the stalk in structure, and a peripheral, more vascular zone which contains the bulk of the neurosecretory material. A nucleus of small nerve cells lies in the junctional region where the stalk joins the main neural process (35).

Pituitary transplants to the kidney of the rat are severely damaged by the transfer, the functional parenchyma of the established grafts apparently being derived from a thin shell of healthy tissue surrounding the massive central infarct. Reorganization of the graft was complete after one week, but normal cytologic differentiation was lost. Large gonadotrophs (PAS+, aldehyde fuchsin negative, AF-) were missing, but smaller ones were found in significant numbers for two to three weeks, after which time they are rarely recognized. Grafts retransplanted under the median eminence suffered greater loss than that at the first operation; however, such grafts obtained at autopsy several months later recovered much of their cytologic characteristics, especially in animals with renewed gonadotropic function (36).

Male albino rats kept on a fat-free diet for 23 weeks revealed that testicular weight is maintained for the first ten weeks, after which progressive testicular degeneration occurs, resulting in failure of spermatogenesis. Pituitary cytology indicated that there is an abundance of FSH- and LH-producing cells after the first nine weeks; thereafter, progressive basophilic changes occurred with an increased number and size of "signet ring" (castration-type) cells. The number of FSH-producing cells gradually decreased and that of LH-producing cells increased until, by the 23rd week, the latter comprised the majority of gonadotropins. Testosterone given after the 20th week results in reversal of basophilic changes (37). Lipemia was produced in the rabbit by injections of ACTH in combination with TSH, LTH, or FSH (38). Pituitaries of gonadectomized obese mice of both sexes, as well as those of nonobese mice, produce excess gonadotropins, but the pituitaries of obese animals elicit less ovarian hypertrophy in the assay animal than those of the nonobese (39). A light-dark synchronized activity rhythm persists in pituitary-isografted C mice. The adrenal cycle probably persists in mice with a significantly altered estrous cycle. Multiple pituitary isografts are associated with adenocarcinoma and adenoacanthomata in virgin female mice of the D<sub>8</sub> and C stocks (40). The pituitary weights of 314 pregnant mice were correlated with the number of uterine implantations: pregnant mice with heavier pituitary glands tend to have a larger number of implantations (41).

*Gonadotropins.*—Isolation and purification of gonadotropins have received renewed interest (42 to 45). The most "highly purified" sheep FSH preparation showed contamination with ICSH (LH), as indicated by repair of interstitial tissue in hypophysectomized female rats and by the increase

in weight of the ventral prostate in immature hypophysectomized male rats (44). Follicle-stimulating and luteinizing hormone from human pituitaries have been isolated with high specific activities. Both FSH and LH induce uterine weight increases in immature rats and mice. Human FSH has approximately the same activity as porcine and ovine FSH. Human LH is ten times more active as pure ovine LH (45). With the human chorionic gonadotropin augmentation test, FSH and LH are detectable in human plasma (46).

International agreement exists for the standardization of human menopausal gonadotropin. Thus far, at least seven preparations have been assayed and up to this time there has been much concern for the methodology of detecting and quantitating them (46 to 48). Using the mouse uterine response, human urinary pituitary gonadotropin (HPG) levels were determined. Single tests performed on 11 normal menstruating women, 14 to 42 years of age, indicate, when positive, HPG levels range from 0.6 to 2.3  $\mu$ gm. of estrone equivalent per 24 hr. (a 3.8-fold span). In 36 per cent of these subjects the values were negative. The pattern of HPG excretion during one menstrual cycle was not constant in the eight women studied. Thirty-seven per cent of the subjects showed a significant peak on the 14th to the 16th days of the cycle (49). Human urinary FSH and LH appear at peak levels at midcycle (50, 51). An assay for FSH in the estrogenized, hypophysectomized rat was proposed (52, 53). A striking increase in ovarian weight is brought about by FSH (53) and LH (54) in previously estrogenized intact and hypophysectomized rats. In intact and ovariectomized rats, stilbestrol caused an increase in pituitary weights (55); thus one should naturally expect intervention by such an exogenously influenced pituitary. Against such a background of the host pituitary, one can expect stimulation of the follicular cells by ICSH (LH), HCG, or estrogen, and functional organization of the theca interna by ICSH (LH), especially in mice [and rats] (56). Compounding further difficulties of assay of gonadotropins in mice is the diurnal variation in response to same (57). Another caution worthy of note is the species-specificity and peculiar reactivity of certain strains of mice to endogenous gonadotropins and age (58). Differences in sensitivities to gonadotropins are also seen in specific alterations in metabolism (59) and in hypercorticism and hyperovarianism (58).

Purified gonadotropins help correct gonadal dysfunction in numerous species: cow (60), rodent (61, 62), and man (63).

Prolactin (LTH), the most obscure of the adeno-hypophyseal gonadotropins, and one which partakes in many bizarre functions, continues to excite the attention of basic investigators. Prolactin levels in hen pheasants rise rapidly during early incubation, with peak levels from the 8th to the 12th days of incubation, and then decline rapidly during latter stages of incubation throughout the first 11 days after hatching (64). Injection of prolactin into pigeons lowers the xanthine-oxidizing activity of liver, kidney, pancreas, and especially the crop sac; it also decreases the amount of uric acid excreted (65). In the killifish, prolactin promotes melanin synthesis

(66). Combinations of prolactin and growth hormone produce a significant increase in the S-35 fixation of the costal cartilage of hypophysectomized male and female rats (67). The oxygen consumption of the mammary gland of rabbits after injection of prolactin was significantly higher than that of the controls (68).

Pituitary prolactin (lactogenic hormone) in rats increases during the first two-thirds of pregnancy, during which time there is a little growth but little secretory activity of the mammary gland. Peak concentration is reached on the 16th day. As the secretory activity of the mammary gland progressively increases during the last one-third of pregnancy, the pituitary levels decrease markedly reaching the lowest point on day 21 (69).

The biology of human chorionic gonadotropin (HCG) was recently summarized (70). Injection of HCG in association with the follicle-stimulating hormone component of the pituitary effects increases in ovarian development (70), uterine weight (71), testicular development and androgen secretion (72), and correction of gonadal dysfunction in a eunuchoid (73).

Testosterone propionate and 19-nortestosterone, both myotropic agents, suppress production or release of pituitary gonadotropins in rats (74). Pregnant mare serum gonadotropin, when incubated *in vitro* with hydroquinones and quinones, loses its gonadotropic activity (75).

#### REPRODUCTION IN THE FEMALE

*Sex cycles.*—Numerous factors influence the sex cycles of different species: endogenous hormones (76), hypothalamic lesions (77, 78), x-rays (79), aging (80), tranquilizers (81), changes in thyroid: serum radioiodide (82), electrical stimulation of sex organs (83), temperature (84), numbers of animals in a given area (85), abdominal electropotentials (86), exogenous hormones (87, 88), genetics, nutrition and body weight (89) seasonal variations (90), and physical abnormalities (91). Changes in the vascular components (92, 93), vaginal cornification, and buccal and urinary smears (94) give further credence to the thought that minute but critical alterations in body fluids are associated with the different phases of the menstrual cycle in addition to the aforementioned (77 to 91).

*Ovary and ovulation.*—Ovarian development and ovulation are under the control of the nervous system and the adenohypophysis (76, 95, 96). The timing of the stages of the maturation divisions, ovulation, fertilization, and the first cleavage of eggs has been thoroughly documented for the mouse (97), rabbit (98), soviet merino lamb (99), rat, and for higher mammals (100 to 105). The electronmicroscopy of the ovum of the rat has been the subject of a most thorough study (103).

Unilateral ovariectomy on the first and second day of the cycle in the hamster results in a doubling of ova released from the remaining ovary. There is no compensatory increase in ovulation after unilateral ovariectomy on the third or fourth day. Injection of PMS or HCG at the time of unilateral ovariectomy on the third day effects a doubling in rate of ovulation in the



remaining ovary (106). Fewer follicles become atretic after division of the oocyte in the immature rat (107). Estrogen retards the loss of developing follicles in the ovaries of hypophysectomized, adult rats (108).

The population of oocytes in the ovaries of the rat decreases slowly and continuously throughout middle and old age. The proportion of oocytes diagnosed as "atretic" declines as the total number of oocytes diminishes (109, 110). The maximum size of the corpora lutea in senile virgin rats exceeds that of the corpora lutea of ovulation of young animals and corresponds to that of pseudopregnancy or midpregnancy. The larger size of these corpora lutea is caused by enlargement of the luteal cells and not by an increase in their number (111). Corpora lutea atretica are present in 40 per cent of pregnant domestic rabbits and account for 7.6 per cent of all corpora lutea. They occur relatively more frequently during the part of the year when rabbits breed most freely. Their formation may be associated with endocrine imbalance since their distribution is not uniform throughout the ovarian population, and is significantly related to an increased loss of ova before implantation (112). Progesterone does not increase the number or size of corpora lutea in pregnant swine (113). The proportion of fragmented ova in unbred gilts increases directly with the postovulatory age of ova (114). Dietary conditions influence the amount of glycogen found in the ova of rats (115). Lipids have been identified during the process of oogenesis in five species of reptiles (116).

Ovarian homografts can survive successfully within millipore filter chambers in the monkey for as long as seven months (117). The histocompatibility requirements of ovarian grafts in mice (118 to 120) and the golden hamster (121) have been reported.

Binuclear ova occur with rather high frequency in submaxillariectomized female guinea pigs. These ova undergo maturation division as in the case of uninuclear follicles. Their origin may be in the fusion of ooplasm of primordial ova (122).

Degeneration of the ova of the rat and rabbit follow the oral administration of 1-(*p*-2-diethylaminoethoxyphenyl)-1-phenyl-2-anisethanol (MER-25) (123).

Various means of ovulation detection in the human have been evaluated; the basal body temperature chart, the endometrial biopsy, and vaginal smears are the most practical for the diagnosis of ovulation. There is as yet, no accurate means of ovulation timing, save a retrospective one. Nevertheless, when used in concert, the methods available provide the clinician with valuable diagnostic aids (124). Ovulation has been induced in hypophysectomized, immature rats with ovine FSH and LH (125) and in hypophysectomized monkeys with porcine FSH and HCG (126). Follicle-stimulating hormone plays an active role in the induction of ovulation in the ewe; the assumption of a specific release of LH may not be valid for this species (127). Progesterone, ICSH, and STH induce ovulation in ovarian fragments of *Rana pipiens* *in vitro*, whereas FSH and prolactin are inactive (128). Com-

binations of sex steroids and subliminal doses of pars distalis extracts induce ovulation in toads (129).

*Epoöphoron and fallopian tubes.*—The epoöphoron of the mouse, guinea pig, and rat lies in the proximity of the hilus ovarii. It is composed of aggregates of tubular bodies, each with a lumen lined by a single layer of epithelial cells. In the guinea pig, the organ is merged by degrees with the rete ovarii in the hilus ovarii, with none of its tubes extended beyond the hilus ovarii into the ovarian tissue. The connective tissue surrounding the walls of the tubes contains no muscle fibers. During pregnancy, the epoöphoron undergoes marked morphologic changes, becoming twisted and meandering. It is very slightly altered in shape in aging animals. The epithelial cells lining the lumen of each tube vary in shape from flat to tall and columnar. Their secretory function is promoted during pregnancy, and the epithelial cells become ciliated in large numbers. The entire organ undergoes extensive histologic change when the animal is sexually mature and becomes pregnant (130). Similar studies on the epoöphoron and paraöphoron of the human have been reported (131). In the ovary of the nine-banded armadillo, several unusual features have been reported: the presence of rete tubules, medullary cords, and occasional testis cords (132).

The acid hematin (Baker's) test permits the identification of two cell types in the oviduct of the rat, one of which is positive for this test and exhibits a secretory cycle (133). Electronmicroscopical studies on the oviducts of rats in estrus reveal that ciliated cells do occur in the epithelium lining the isthmus of the Fallopian tube. The only other type of cell identified in this region was one containing secretory granules with numerous long, thin protoplasmic processes projecting from their free border. The number of alpha-cytomembranes in the secretory cells is larger than the number of these membranes in the ciliated cells. The cytoplasm of the secretory cells contains vesicles; the view is expressed that the secretory granules arise within these vesicles (134). Similar studies on the mouse fallopian tube indicate that the images of proper cilia, in which only one central filament is observed, are thought to be unresolved images of two central filaments caused by the geometrical thickness of ultra-thin sections (135).

In the rabbit fallopian tube, the PAS reaction applied to freeze-substituted plastic-embedded tissue reveals the presence in ciliated cells of homogenous cytoplasmic inclusions which are diastase-digestible. In secretory cells a homogenous diastase-resistant structure is seen surrounded by a layer of secretory granules. Structures probably concerned with the so-called ciliated vesicles are observed in basal cells. Daily administration of 15 or 20  $\mu$ g. estradiol benzoate to ovariectomized rabbits induces mitotic proliferation of the epithelium, a maximum being reached after three to four days which corresponds to the phase of logarithmic growth when applied to the growth curve. After four days treatment with 15 to 20  $\mu$ g. estradiol benzoate daily, it appears that (a) the relative number of nonciliated cells within certain limits increases with estrogen dose, but the relative number of

secretory dividing cells is not significantly changed; (b) a correlation exists between the relative numbers of nonciliated cells and secretory cells after treatment with 10 and 15  $\mu$ g. estrogen daily; (c) the regression of this correlation reveals a predominance of secretory dividing cells in relation to the number of nonciliated nondividing cells. This evidence signifies that the secretory cells of this epithelium regenerate independently of the ciliated cells, and contrasts with the transformation theories of earlier reports (136).

Histochemical observations on the epithelium of human fallopian tubes reveal that (a) glycogen is diffusely present in the ciliated cells and basally in the secretory cells during pregnancy; (b) lipids are present in the cytoplasm in the form of granules and in borders of vacuoles; (c) alkaline phosphatase is present along the surface of the secretory cells; (d) nonspecific esterase is found in cytoplasm of the secretory cells, and (e) PAS-reactive material is encountered in the apical parts of the secretory cells (137). Positive Sudan III material (lipoid droplets) is identifiable in the epithelium, muscular coat, and interstitial components of the human tube (138). Each of the three layers of the tubal wall continues into the uterine wall without interruption. The inner layer of the uterine wall consists of four systems of muscle bundles. This structure enables the muscular coat not only to exert a constrictor effect on the largest part of the intramural tube, but also to influence the transportation of the ovum in the direction of the uterine cavity, and that of the sperm in the opposite direction, by means of peristaltic waves (139).

In studies on pressure changes in uterotubal insufflation in rabbits, the unavoidable conclusion that the uterus has nothing whatever to do with fluctuations in pressure during uterotubal insufflation appears dominant (140).

Upon post-mortem examination of 20 white Plymouth Rock laying hens, it was found that 80 per cent possessed persistent right oviducts 16 cm. or more in length. These hens also possessed normal, apparently functional left oviducts and ovaries. Each persistent right oviduct exhibited vascularity and color associated with functional oviducts (141). Albumen precursors are formed in at least two steps in the oviduct magnum (albumen-forming segment of the oviduct of the hen). A large part, perhaps 45 per cent, of the albumen deposited by the magnum appears to be synthesized and transferred at the time of deposition (142). Administration of 1 mg. progesterone daily significantly depresses the weight and dry matter contents of the hypertrophied oviducts of estrogenized-androgenized immature pullets and normal hens (143).

*Uterus and cervix.*—Electronmicroscopical investigations of the human endometrium reveal that in the follicular phase the glandular cells contain many small mitochondria; in the pro gravid (formerly called "secretory") phase, the mitochondria become polymorphous and vacuolated and their cristae are very irregular (144). Marked improvement in endometrial cytologic patterns follow the use of 17 $\alpha$ -hydroxyprogesterone caproate (Dela-

lutin), particularly in amenorrheic patients who have been ill less than eight months (145). A combination of hormones and changes in vascular patterns best explains the underlying events leading to menstruation (146). The guinea-pig uterus and strips of human myometrium are stimulated *in vitro* by ether extracts of menstrual fluid (147). Patients who deliver prematurely show a reduction in urinary estrogen or pregnanediol excretion, or both, and have an excessive uterine contractility when compared to normal patients. When pregnancy of any gestational length is complicated by toxemia, hemorrhage, infection, or twins, a reduction in myometrial clearance of radioactive sodium results. Low placental hormone secretion and excessive uterine contractility are seemingly related to a reduction in uterine circulation, especially in cases of toxemias of pregnancy (148). Ovarian stromal hyperplasia or functioning ovarian tumors in five of seven cases may account for persistence of postmenopausal endometriosis; in one case, adrenocortical estrogen appears suspect (149).

The uterine artery of Sprague-Dawley rats grows from a circumference of 100  $\mu$  at birth to 825  $\mu$  at three months (150). Uterine growth in pregnancy comprises muscular hypertrophy and an extensive increase in connective tissue ground substance as well as an increase in fibrillar elements. There is little evidence of muscular hyperplasia (151). 2,4-Dichlorophenol and other phenols catalyze the aerobic oxidation of reduced pyridine nucleotides to the oxidized form by homogenates of rat uterus (152). Swiss mice, 21 days old, given single subcutaneous injections of either 10  $\mu$ g. estradiol propionate, 1.0 mg. testosterone propionate, or 1.0 mg. progesterone and necropsied 72 hr. later were studied for uterine water, protein, lipid alkaline phosphatase,  $\beta$ -glucuronidase, ascorbic acid, cholesterol, and glycogen. Estradiol influences all components except cholesterol; testosterone propionate failed to alter alkaline phosphatase and cholesterol; progesterone had no effect on lipid. During the estrous cycle,  $\beta$ -glucuronidase exhibited only minor variations. Human chorionic gonadotropin administered for 20 days produced effects similar to those of progesterone (153). A generally high diphosphopyridine nucleotide-diaphorase (DPN) activity is found in all components of the uterus during the "ballooned" or proestrous stage in the rat. The luminal epithelium reacts most intensely, while the uterine glands react less intensely. A drop in DPN activity follows the peak activity of proestrus. Similar reactions are seen with the localizations for succinic dehydrogenase (154).

Fructose 1,6-diphosphate has a restorative and stimulating action on uterine fibers in the pregnant guinea pig (155). Electrophoretically homogeneous actomyosin has been prepared from pregnant rabbit uterus (156). Mobilization of leucocytes occurs more rapidly in the uterus of the estrous rabbit. A significant negative relationship exists in estrous animals between bacterial numbers within the uterus after experimental uterine infections (157).

The effects of hormones on uterine growth continue to receive a great deal of attention (153, 158, 159). Growth hormone (STH) induces an increase

in uterine weights in both hypophysectomized and hypophysectomized-ovariectomized rats; it exerts a direct effect on the uterus, as well as an indirect one through the ovary (160).

Using the Corner-Allen progestational proliferation index and endometrial mitotic counts in rabbits, it was found that the biological effectiveness of intravenously administered progesterone is approximately 25,000 times less than that of the intrauterine administration of progesterone and 25 times less effective than use of the intramuscular route (161). Labelled hexestrol appears selectively in the uterus and in other organs which respond to estrogen (vagina, mammary glands, ovaries, oviducts, and the pituitary), as well as in the kidney, liver, and intestine (162). Progesterone effects a significantly higher resting potential (63.8 mv.) than does estrogen (56.7 mv.) in single uterine muscle fibers in the rat (163). Uterine noradrenaline levels in the rat are increased one hour after injection of 100  $\mu$ g. doses of epinephrine subcutaneously (164). Magnesium ions augment the response of the rat uterus to the U.S.P. standard, oxytocin, lysine, and arginine vasopressins, and all the analogues tested, but there is considerable variation between substances in the degree to which this potentiation occurred (165).

From an examination of 853 unselected uteri, the patterns of behavior of the squamous epithelium and of the cervical mucosa at their junction were established. The position of the histologic internal os (upper limit of the cervical mucosa) shows a close relationship to that of the lower limit of the cervical mucosa. When the volume of the cervical wall increases, the lips of the portio protrude, carrying with them the cervical mucosa (eversion). At the same time the upper limits of the mucosa move downward (166). In a study of 267 histologic sections of the squamocolumnar area of the cervix uteri obtained from premature babies, newborns, children, pregnant and nonpregnant women, and postmenopausal women, an abrupt squamocolumnar junction was noted in only 29 per cent of the sections, and in 71 per cent there was a zone of transition between the columnar and squamous epithelium (167). The numerous cervical factors in reproduction were recently reviewed extensively (168).

X-ray diffraction shows that dried cervical mucus "ferning" in the pregnant human female is composed of true crystals which are a mixture of sodium chloride, potassium chloride, and a small amount of organic matter (169). Endocervicitis influences fern formation (170). The specific viscosity of the sample of cervical mucus isolated from cows in estrus is about twice that of samples from pregnant cows, indicating that the estrous mucoid is more expanded than the pregnancy mucoid (171). Changes in cervical secretions typical of estrus and pregnancy can be duplicated in ovariectomized cows with estrogen and progesterone (172). Arborization of cervical mucus in the ewe remains as a reliable test for diagnosing the different phases of the cycle (173). As in the cow, graded amounts of estrogen and progesterone can duplicate the different phases of the cycle in the ewe (174).

*The vagina.*—The embryology of the vagina is of special interest for sev-

eral reasons, including the possible origin of carcinoma of the cervix uteri in the epithelium of the vaginal portion of the cervix (portio vaginalis). Associated with "female pseudohermaphroditism" is absence of the lower portion of the vagina. The original vaginal epithelium is derived from fused Müllerian ducts. During the prenatal period, it is replaced by entoderm from urogenital sinus or by ectoderm from proctoderm. The Müllerian ducts develop in a monkey embryo of 36 days, and in human embryos of 37 days' ovulation age (175). In rats, the Müllerian cylindrical epithelium changes to squamous epithelium, probably through a process of metaplasia (176). The vagina of the immature mouse can be cultured in biological and completely synthetic media for periods of up to 72 hours. Growth and cornification of epithelium occur in absence of estrogens and are not prevented by addition of a number of hormones and vitamins to the medium (177). Vaginal explants from prepuberal rats can be cultivated for periods of one to six days on a synthetic medium. Vaginal epithelium in the medium alone keratinizes after two days *in vitro*. Addition of approximately 0.08  $\mu$ g. estradiol-17 $\beta$  induces precocious development of a cornified epithelium in one day. Addition of 0.3  $\mu$ g. vitamin A inhibits but does not prevent keratinization *in vitro* (178).

The gross and microscopic anatomy and innervation of the human vagina have been studied with those of the uterus (179, 180). The proton resonance spectra and relaxation times of vaginal cell sediments of the human were followed during 20 cycles. The position of the proton resonance signal was localized on the negative side of that for water and shows a tendency to increase from about -0.10 p.p.m. postmenstrually to about 0.02 p.p.m. (water reference) at the height of the luteal phase. The  $T_1$  values are around 0.6 to 1.0 sec. during the entire menstrual cycle, while the  $T_2$  values are about 0.03 sec. at the beginning of the menstrual cycle, with a tendency to increase to 0.10 as the cycle reaches the height of the luteal phase (181). Macht's findings in 1918 and 1928 on the ready absorption of a variety of chemicals from the vagina are confirmed and extended, especially since it can be demonstrated that there is a unidirectional transmission of substances from the vagina into the blood stream, with no such transmission in the reverse direction (182).

The basal cell of the human vaginal smear may have a different appearance under certain circumstances. The cytoplasm may be more dense, more basophilic, and may contain fine vacuoles. This phenomenon was first observed in patients with cancer of the uterine cervix before treatment. When these cells are numerous, the patient usually responds favorably to radiotherapy; when few, salvage rate is poor. Because the presence of these cells seemed to indicate that the patient and tumor are particularly vulnerable to radiation, or were sensitized, they were referred to as sensitization-response cells. It was shown that the sensitization response is a cellular immune response. It can be elicited by a variety of antigens but not by nonantigenic irritants (183).

Histochemical analyses of the vagina reveal the presence of PAS+protein-bound sulfhydryl and disulfide groups in the human fetal vaginal epi-

thelium (184). Estrogens effect intense depositions of alkaline phosphatase and mucopolysaccharides in the uteri and vaginae of mice (185). Vaginae of nonhormonally treated rats contain minimal DPN activity and no histochemically demonstrable succinic dehydrogenase activity (SDH). The DPN system appears to respond more readily to estrogen and progesterone, whereas succinic dehydrogenase activity is mostly low or absent (186). DPN is completely absent in mouse vaginal cornified cells, but highly intense in cells with less cytodifferentiation (187). Strain differences exist in the sensitivity of vaginal cornification response of ovariectomized mice to injected or topically applied estrogens (188). Rats aged 17 months or more do not undergo regular estrous cycles. The response of the vaginal epithelium to estrogen does not appear to be less in senile than in young rats (189). In tests of vaginal cornification, anti-estrogenic activity has been demonstrated in dimethyl-, ethyl-, and *n*-propylstilbestrols. Dimethylstilbestrol, the most potent anti-estrogen, inhibits the increase in vaginal mitosis and epithelial thickness, which normally follows administration of estrone to ovariectomized mice (190).

*Estrogens.*—Estrone, estradiol, and estriol appear in human pregnancy plasma (191). Estriol and estrone have been isolated and identified in extracts of bile obtained by duodenal intubation from 17 Rh-sensitized healthy pregnant women at term. Presumptive evidence indicates that 16-epiestriol and estradiol-17 $\beta$  are also present (192). Stallion testis, human term placenta, human fetal liver slices, and human feminizing adrenal cortical carcinoma can convert C<sup>14</sup> testosterone to C<sup>14</sup> estrogenic steroids (193, 194). Ovaries of high and low mammary tumor strains of mice convert C<sup>14</sup> testosterone to C<sup>14</sup> estradiol-17 $\beta$  equally well (195). 18-Hydroxyestrone (196), 2-methoxyestrone, and 2-methoxyestradiol (197) have been isolated from the urine of pregnant women, as have 16 $\alpha$ -hydroxyestrone and 16 $\beta$ -hydroxyestrone (198). Estrone serves as the principal, if not the exclusive, substrate for hydroxylation at C-16 to give both the alpha- and beta-hydroxy compounds (199). Administration of ACTH produces a three- to ninefold increase in output of urinary estrogens in women with breast cancer; adrenal-ectomy completely abolishes this response (200).

The *in vitro* distribution of C-14 and H-3 labelled estrone, estradiol-17 $\beta$ , and estriol between erythrocytes and serum of horse blood reveals that 30.2  $\pm$  1.1 per cent estrone, 20.7  $\pm$  2.7 estradiol-17 $\beta$ , and 37.3  $\pm$  3.0 per cent estriol radioactivities are associated with erythrocytes at 50 per cent hematocrit (201). The enzymatic transport of hydrogen by estrogenic hormones is well established (202 to 206).

The metabolic and extragonadal effects of the estrogens are numerous and diverse. Uterine cells do not exclude D-xylose-1-C<sup>14</sup> in the absence of estradiol (207). Partial hepatectomy does not modify growth response of the uterus to estrogen (208). Specific effects are obtained with specific estrogens (209 to 212). The physiologic and pharmacologic effects of estriol are reviewed extensively (211). Stilbestrol additions to legume-free rations fed to



gilt lower the ovulation rate, corpora lutea formation, and number of live young (213). The uteri of spayed rats show significant reduction in histamine content and concentration after single intramuscular injection of 2  $\mu$ g. estradiol, estrone, or estriol (214). Estradiol valerate induces endosteal ossification in autogenous transplants of mice tibiae (215). Thyroxine and estradiol valerate synergize in the inhibition of linear growth in the mouse femur (216). Kinetic studies, in agreement with observations made by autoradiography, indicate a reduction of approximately 50 per cent in the rate of resorption in upper ends of the tibiae of estradiol-valerate-treated rats (217). Estrone accelerates the rate of calcium accretion and tends to enlarge the size of labile bone mineral pool in mice. Parathyroid extract diminishes the accretion rate but has no effect upon calcium exchange. Simultaneous administration of both hormones effects an antagonism between them, one which is presumably dependent on changes of metabolic processes associated with formation of new bone crystal and growth of established crystal (218). Atherosclerosis in cockerels results from estradiol cyclopentylpropionate dosage levels (2.5 mg.) lower than those required to produce hypercholesterolemia and other blood serum changes associated with atherosclerosis (219).

Thyroxine decreases and methylthiouracil increases vaginal reactivity to estrone, a confirmation of Bigger's and Claringbold's findings (220). A dose of 3.6  $\mu$ g. per day of estradiol benzoate given to female rats causes a significant increase of 35.5 per cent upon thyroxine secretion rate over the average control value of 1.24  $\mu$ g. per 100 gm. per day of 1-thyroxine, while 1, 15, or 50  $\mu$ g. of estradiol benzoate had no effect (221).

*Progesterins and related ovarian steroids.*—17 $\alpha$ -Hydroxyprogesterone and  $\Delta^4$ -androstene-3,17-dione have been identified in extracts of seven human follicles, obtained one to two days before ovulation, 41 corpora lutea of the menstrual cycle, and 19 corpora lutea of pregnancy (222). With the Hooker-Forbes test, it was found that progesterone concentration increases gradually in human pregnancy, blood plasma ranging from 6 to 26  $\mu$ g. per cc. Maximal values are reached at the 26th week, continue until the 32nd week, then decline toward the end of pregnancy (223). When the blood level of progesterone in the human is determined chemically, there appears to be a steady rise in blood levels from the 11th to the 35th week, but they thereafter rise more rapidly, and remain high during labor (224). The amniotic fluid of 22 monkeys contains 0.19 to 1.70 free progesterin per ml. (225). Progesterone and 20 $\alpha$ -hydroxypregn-4-en-3-one have been identified in plasma and in the placenta of intact and ovariectomized ewes (226, 227). Progesterone and 17 $\alpha$ -hydroxyprogesterone have been identified in follicular fluid, in peripheral blood of pregnant and nonpregnant mares, and in umbilical cord blood collected during foaling (228, 229). Progesterone and 4-pregnen-20 $\alpha$ -ol-3-one are also present in the ovaries, blood, and fat of pregnant rats (230, 231).

Six metabolites of progesterone have been isolated after incubation with human liver suspensions: 5 $\alpha$  and 5 $\beta$ -pregnane-3:20 dione, 3 $\alpha$  and 3 $\beta$ -hydroxy-5 $\alpha$ -pregnane-20-one, 3 $\alpha$ -hydroxy-5 $\beta$ -pregnane-20-one, and 5 $\beta$ -

pregnane-3 $\alpha$ :20 $\alpha$  diol (232). Three pathways for excretion of progesterone metabolites in human are the urinary, gastrointestinal, and respiratory tracts (233). Progesterone disappears rapidly from oily solutions injected subcutaneously or intraperitoneally in mice (234, 235). It has been estimated that the half life of progesterone in the human is less than five minutes (224).

Progesterone and 17 $\alpha$ -hydroxyprogesterone caproate (Delalutin) maintain pregnancy in ovariectomized rabbits (236) and in habitual aborters (237).

Masculinization of the female fetus has been reported after administration of progesterone, 17 $\alpha$ -ethynyl-19-nortestosterone, and 6 $\alpha$ -methyl-17 $\alpha$ -acetoxyprogesterone to pregnant rats (238) and after medication with progesterone, 19-nor-17 $\alpha$ -ethynyltestosterone, norethynodrel, 17 $\alpha$ -ethynyltestosterone, and methyltestosterone to pregnant human females (239, 240). The biological activities of a number of 6-methylated progesterones reveal that the 6 $\alpha$ -methyl group increases potency over the parent compound; 6 $\beta$ -methylation of 17-acetoxyprogesterone increases potency to a lesser extent (241).

Progesterone administration to rats and dogs with experimental hypertension and to people with primary hypertension results in a decline in blood pressure levels (242). A thermogenic response to progesterone is obtained in cows (243). Progesterone inhibits the estradiol-induced decrease in uterine carbonic anhydrase levels, but by itself is without effect (244).

*Fertility and sterility.*—The recent literature emphasizes the numerous and stubborn causes underlying sterility. These include psychic phenomena (245 to 248), inadequate and unsatisfactory modes of living (249), ovulation difficulties (250), gonadal dysgenesis (251), bilateral cystic ovaries (252), x-ray exposure (253 to 255), luteal insufficiency (256, 257), endometrial difficulties (258 to 260), incompetent cervix (261 to 264), vaginal infections (265, 266), dietary deficiencies (267, 268), obesity (269), diabetes mellitus in men (270), and sperm abnormalities (271, 272).

Sterility appears to be corrected by minimal and intensive therapy (273), psychological counseling (274), psychotherapy (275), ovarian wedge resection in polycystic conditions (276), conservative and plastic surgery in ruptured ectopic pregnancy (277), administration of vitamin E (278), cortisone therapy (279, 280), standard diagnostic procedures and reassurance (281, 282), and hormonal manipulation (283).

*Fertilization.*—The fertilization process, including (a) the penetration of the spermatozoon into the egg, (b) formation of a spermatozoon, the male pronucleus, and an egg nucleus, the female pronucleus, (c) growth and development of pronuclei, (d) replacement of pronuclei by chromosome groups, and finally (e) the union of two chromosome groups, is beautifully and extensively summarized (284).

Fertilization and deposition of the mucoprotein coat of ova can occur in ovariectomized rabbits. A low level of estradiol benzoate increases rate of spermatozoon passage, or retention of the ova in oviducts. Contractility of

the uterus, tonic restrictiveness of the cervix and uterotubal junctions, and secretion of mucus vary considerably in sensitivity to estrogen. In ovariectomized rabbits, approximately one  $\mu\text{g.}$  of estradiol benzoate induces physiological equilibrium resembling estrus, but not normal transport function. The role of progesterone and combinations of progesterone and estrogen in such phenomena awaits experimentation and elucidation (285).

*In vitro* experiments with human sex cells reveal that sperm penetrate the membrane of the ovum 2 hr. after insemination; after 4 hr. a great number of sperm, with head and tail or head only, could be seen in the perivitelline space and cytoplasm of the ovum. Conjugation of male and female pronuclei was observed 15 hr. after insemination. Segmentation began after 20 hr., and after 26 hr., 3 blastomeres of different sizes were recognizable (286).

Electronmicroscopical studies indicate that the individual spermatozoon of *Hydroides hexagonus* (ANNELIDA, Polychaeta, Sedentaria = annelid worm) forms a hole in the vitelline membrane by lysis (287, 288). Fertilization takes place 10 to 30 min. after ovulation in hens (289). Kinetic (290) and immunologic (291) considerations may yield much important information regarding fertilizing ability of sperm.

In artificial homologous inseminations in the human, it has been determined that the greatest success rate follows intrauterine insemination. No pregnancies resulted from specimens with average counts below 40 million per cc., with sperm motility below 40 per cent, or with abnormal forms above 30 per cent (292).

*Pregnancy and placenta.*—The vital role of the trophoblast in maintaining life *in utero* and ensuring the best possible ecology for successful development of new life is the subject of two extremely beautiful and scientific essays (293, 294). The biology of HCG suggests that it has an important role in pregnancy (70). Caution against improper and undue use of HCG is stressed, especially since it may cause a marked rise in A and B antibody titer (295). A further plea is made for the re-examination of the use of progestins and all other drugs in the course of pregnancy. The steroid hormones should find a sounder basis for their use in the expectant female than they presently enjoy. The incidence of masculinization of the female infant by progestins, or estrogens, warns stoutly against indiscriminate practices (238 to 240, 296). Progesterone, as well as esterified, structurally modified, and halogenated derivatives thereof, currently demonstrates some measure of success in habitual and chronic aborters and in toxemias of pregnancy (237, 297). Considerable and very rigid experimentation with highly standardized controls that lend to complete duplication, quantitation, and positive signs of helpfulness is yet required for many of the progestins on the market. Witness the very harmful and undesirable masculinizing effects induced by many of the currently available oral progestins (238 to 240, 296). Basic scientists are still experimenting and trying to ascertain the biologic effects of the naturally occurring hormones in reproductive processes (298 to 305).

Our knowledge of posterior lobe hormones seems to rest on a firm founda-

tion. No one doubts the ability of oxytocin to induce rhythmic contractions in the estrogen-dominated uterus of many species, and no one doubts that parturition is a complex process in which uterine contractions constitute only one of several participating mechanisms (306). Synthetic oxytocin is seemingly more efficacious in conduction of the third and fourth stages of labor than ergot alkaloids (307).

Oxygen dissociation curves of the bloods of nonpregnant and pregnant sheep and goats have been calculated and established. In the nonpregnant uterus the blood flow is approximately 25 ml. per min. It increases to approximately 200 ml. per min. at the 80th day of gestation and exceeds 1000 ml. per min. near the end of the 150-day gestation period of these animals (308, 309). With the use of indwelling catheters, studies of blood samples from an artery and one or more uterine veins of unanesthetized pregnant ewes at selected stages in gestation reveal that the pH of arterial blood ranges from 7.40 and 7.62 (avg. 7.47), and its CO<sub>2</sub> tension between 30.4 and 42.2 (avg. 33.5). The coefficient of oxygen utilization by the uterus increases from less than 10 early in gestation to 30 and above in the last third of gestation, 145 to 147 days (310). The CO<sub>2</sub> tension is higher in the blood of the fetal sheep or goat than it is in the maternal; this relationship is the basis for the assumption that CO<sub>2</sub> produced by the fetus reaches the maternal blood by transplacental diffusion. New evidence provides, for the first time, information that the CO<sub>2</sub> tension in fetal blood is higher in "steady state" conditions (311).

Evidence indicates that there is a single 17 $\beta$ -hydroxysteroid dehydrogenase in the placenta which catalyzes estrogen-dependent transhydrogenations. 17 $\beta$ -Hydroxysteroid dehydrogenase has the same stereospecificity for pyridine nucleotides as the transhydrogenase reaction (204, 313). Students of placentalogy will find a recent historical account of stimulating interest (312).

*In vitro* conversion of 16-ketosterone in the human placenta results in compounds characterized as estriol and 16-ketoestradiol (314). 16-Oxo-estradiol-17 $\beta$  appears in the human placenta (315).

The urinary HCG titration test is recommended for diagnosis of placental insufficiency and fetal death in early pregnancy. Fluctuations in urinary elimination of estriol indicate dysfunction of the placenta (316).

Relaxin remains as the most elusive of the 14 hormones concerned with human reproduction. Additional controlled observations are required to clarify its usefulness in treatment of dysmenorrhea, threatened premature labor, and in shortening labor (317 to 320). In rats, relaxin (10 guinea-pig units) causes a uterine water increase from a control value of 80.2 per cent to 84.2 per cent. Adrenalectomy effects a four- to sixfold increase in sensitivity to relaxin and an increase in magnitude of the response; the action of relaxin in this response is modified by ovarian and adrenal steroid hormones (320). The resultant depression of thyroid levels after administration of thiouracil does not impart irreparable damage to the action of relaxin in pelvic relaxa-

tion in mice. A large single dose of low-potency relaxin is capable of producing symphyseal separation even in the presence of thyroid block (321).

Purification of nonsteroid hormonal fractions continues to ensure a more complete picture as to the number of different polypeptides with relaxin activity (322, 323). Combinations of different polypeptides with relaxin activity may well be the best treatment for many of the stubborn obstetrical problems.

*Mammary gland and lactation.*—Using DNA as an index of mammary gland growth in pregnant and lactating mice, it was determined that 30.6 per cent of total growth occurs during the first 12 days of pregnancy, 45.7 per cent in the latter half of pregnancy, and 21.9 per cent further proliferation from parturition to day 14 of lactation (324). In the rat there is a 41.5 per cent increase in mammary gland growth between the 18th to 20th day of pregnancy and day 5 of lactation. Marked glandular proliferation occurs following parturition, 5 to 10 days (325). Daily doses of 1.0  $\mu$ g. estradiol benzoate and 2.0 mg. progesterone to mature ovariectomized rats for 19 days induce mammary gland growth (mean total DNA/100 gm.) comparable to that of rats pregnant 18 to 20 days (326). Daily administration of 2.0  $\mu$ g. estradiol benzoate plus 6.0 mg. progesterone to mature ovary-thyroparathyroidectomized rats for 19 days results in mammary gland growth (DNA) comparable to that of ovariectomized rats receiving the same treatment (327). Glands of ovariectomized rats receiving 1.0  $\mu$ g. of estradiol benzoate, 3.0 mg. of progesterone and 3 to 6  $\mu$ g. per 100 gm. of thyroxine did not differ from controls. Doubling the estrogen and progesterone levels induces a significant increase in DNA (328). In gonadectomized, hypophysectomized male and female rats, combinations of testosterone and growth hormone produce extensive alveolar development. Testosterone and prolactin stimulate marked secretion but do not produce clear lobule-alveolar development (329). In hypophysectomized-ovariectomized-adrenalectomized rats, growth of mammary ducts can be induced with estrone, growth hormone, and corticoids; lobule-alveolar development ensues if in addition to the above combination progesterone, plus prolactin, is given (330). In intact and ovariectomized rats and in intact, ovariectomized, and ovariectomized-hysterectomized mice, combinations of relaxin and estrogen induce significantly stimulated growth of the lobule-alveolar system (331, 332). Daily subcutaneous injections of 20  $\mu$ g. of reserpine or 0.5 mg. of serotonin creatinine sulfate base/kg. body weight in mature virgin rats after administration of 10  $\mu$ g. estradiol for 10 days initiates milk secretion in 14 of 15 rats and induces growth of lobule-alveolar tissue (333). Prolactin stimulates increased oxygen consumption in the mammary glands of mice and rabbits (334, 335).

It has been proposed that in rats suitably prepared with estrogen and progesterone, elevation in succinoxidase-cytochrome oxidase and in arginase might serve as endpoints for lactogenic and lactopoietic assay, respectively, of hormone preparations (336).

Prolactin induces lactation in cases of hypolactation and in ovariectomized

and sterile women (337, 338). Lactation can be induced by reserpine in New Zealand White breed, but not in Dutch rabbits (339), by prolactin in intact and hypophysectomized rats (340 to 342), by providing new litters to female rats every 10 days (343), by electrical stimulation of uterine cervix of rats (344), and by injections of hypothalamic tissue to rats (345). Successful lactation depends upon intact and nondisturbed hypothalamic nuclei (346).

Mammary gland involution in rats can be retarded by prolactin (347), reserpine (348, 349), valyl oxytocin (350), and by acetylcholine injections (351).

#### REPRODUCTION IN THE MALE: TESTIS AND MALE REPRODUCTIVE TRACT

From a group of 305 human testicles, 225 of which were either post-mortem or surgical specimens, it was calculated that (a) the mean weight of testicles is 19.7 gm.; (b) when only testicles exhibiting efficient spermiogenic activity were taken into account, the mean weight is 21.5 gm.; (c) the smallest testicle with good spermiogenesis activity weighed 9 gm; (d) comparisons restricted to testicles with good spermiogenic activity do not reveal any statistically significant differences between mean testicular weight of younger age group (under 50) and that of the older one (above 50); (e) Leydig cells are more numerous in younger age groups and diminish with rising age; and (f) testicular biopsies with very few exceptions reflect very closely the histological appearance of the whole testicle. This same report on testicular morphology contains a histopathological study with special reference to biopsy findings in hypogonadism with endocrine disorders and in gynecomastia (352).

Studies on the development of the Leydig cells in the rat from day 15 of fetal life until two months after birth reveal at least two phases of growth and differentiation and one of regression and dedifferentiation. The most conspicuous turning points are a climax of growth on fetal day 19 and a low period of regression on day 4 after birth (353). The nuclear volume of the interstitial cells in mice is highest toward midnight, decreases until 6 a.m., remains at same level until noon, then continues to decrease, reaching the minimum at 6 p.m. This is followed by an increase until the 24-hr. maximum is reached (354).

Aldosterone monoacetate arrests the development of rat testes as measured by low testicular weight and cessation of spermatogenesis at the primary spermatocyte stage. Leydig cell differentiation is also inhibited, but the interstitial elements show no atrophic changes (355). Intratesticular introduction of cholesterol and stigmasterol in adult rabbits results in a considerable weight increase in the injected testicle, an enlargement of the interstitial gland, and increased production of spermatozoa and steroid hormones (356). Cooling of mice to 1-2°C. during irradiation afforded considerable protection to testes (357). Triethylenemelamine (0.05 mg./kg. five times weekly for four weeks) induces subfertility or infertility in male rats; a dose of 0.2 mg. per kg. daily for five days produced, in addition to subfertility and infertility,

a destruction of spermatogonia followed by a maturation depletion of germinal epithelium (358). Similar effects have been observed in mice (359). Daily administration of 2 gm. griseofulvin to 14 normal men for three months did not produce significant changes in semen quality or in histology of testes of eight of the males (360).

Two cases of testicular feminization have been described, one typical and the other intermediate between testicular feminization and gonadal dysgenesis (361).

A critical review on epididymal structure and function discusses spermatozoa ripening (362). The bio- and histochemistry of the epididymis of the mouse has been extensively investigated (363 to 365).

*Sperm.*—Two excellent treatises dealing with electronmicroscopic investigations of human sperm have appeared (366, 367). Viewed in phase contrast, dried, unstained spermatozoa fall into two distinct populations regarding head and nuclear size, shape, diffraction of light, and chromosomal pattern. The relative sizes and shapes of chromosomes in the center of the spermatozoon nuclei indicate that the smaller heads contain the Y and the larger the X chromosome (368). The serum of about 2000 male partners of sterile couples has been investigated; about 3 per cent appeared to have sperm-agglutinins in the serum in titers of 32 or higher; this was never the case in 416 fertile men (369).

Morphological and physiological studies on the sperm of monkeys (370), bulls (371, 372), rams (373, 374), swine (375), laboratory mammals (376), and frogs (377) have been reported. Chemical studies are equally numerous but not as extensive (378 to 385). The report by King & Mann (378) is by far the most outstanding contribution of the year in this area. Sorbitol was identified as a chemical constituent of the seminal plasma in the ram, rabbit, bull, boar, stallion, cock, and human male. In intact spermatozoa the steady state of reversible enzymic conversion of sorbitol to fructose depends upon the actual ratio between oxidized and reduced DPN. It is suggested that the DPN ratio within the sperm cells themselves may depend upon the respective levels of fructose and sorbitol in seminal plasma (381). "Cold-shock" treatment of ram (378, 385) and bull (385) spermatozoa rapidly immobilizes them.

A technique of merit has been presented for the objective and quantitative measurement of sperm motility based on the ability of spermatozoa incubated at 37°C. to migrate from a chamber (12 mm. in diam.) in a lucite block containing 0.5 cc. of diluted semen through an interposed wire screen 10 $\mu$  mesh) to an identical chamber containing sperm-free fluid (386). Spermatozoa of low motility from 14 oligospermic semen samples showed an average increase of 30 per cent motility when suspended in seminal plasma of normal semen, the maximal increase occurring between two and four hours. Conversely, seminal plasma of oligospermic semen decreased motility of normal semen by 40 per cent in 17 of 21 experiments (387). Spermatozoa travel in the female genitalia by their own active movement, and passively through forces



produced by the genital tract as observed experimentally and clinically (388). Seminal fluid instilled into the human vagina, cervix, or uterine corpus possessed the property of influencing the motility of the uterus. The deposition of seminal fluid in the vagina alone results in contraction of the corpus (389).

Pathways emanating from the hypothalamus are potentially capable of exerting profound effects on the contractile activity of both male and female genital tracts. Whether these mechanisms operate in intact animals to influence the efficiency of sperm transport has not been unequivocally demonstrated (390).

A normal or decreased number of Leydig cells was found in 26 of 32 elderly men with prostatic carcinoma; hyperplasia was present in only two patients, an incidence of 6 per cent. Estrogen therapy before orchiectomy produced a decrease in number of interstitial (Leydig) cells, but does not predispose to interstitial cell hyperplasia (391).

Luteotropin alone has no effect on the seminal vesicles of orchiectomized guinea pigs but, in combination with subminimal amounts of testosterone propionate, caused a significant increase in weight of seminal vesicles as well as the height of epithelium (392). Seminal vesicles of orchiectomized rats contract spontaneously *in vitro*. Testosterone propionate given before necropsy inhibits contractions; injection of water-soluble androgen *in vitro* has the same effect. Seminal vesicles of orchiectomized rats have lower thresholds to epinephrine, and possibly to acetylcholine, but not to noradrenaline. Thresholds to acetylcholine but not to noradrenaline are elevated after injection of water-soluble androgen into *in vitro* chamber. Androgen and the autonomic nervous system probably interact at or near the cell membrane of the vesicle musculature (393).

*Androgens.*—Testosterone has been isolated and identified in systemic blood of normal male subjects given HCG intramuscularly, but could not be found in non-HCG-treated normal males (394). Testis of a gonadotropin-stimulated stallion, when perfused with horse blood containing sodium acetate-1-C<sup>14</sup> for 24 hours, yielded the following radiochemically pure substances: 4-androstene-3,17-dione, testosterone, progesterone, and 17-hydroxyprogesterone (395). The gonadal response to HCG may serve as an indicator of testicular activity (396). Tubular lipids of pigeons probably contain progesterone and its metabolites. The testis of "prenuptial" birds contains androgen, but no progestins (397). Excretion of radioactivity of androgenic and progestational 4-C<sup>14</sup> steroids in the bovine male and female followed a relatively smooth pattern; however, a hyperbolic curve was observed in only one case in an animal given non-esterified testosterone-4-C<sup>14</sup> (398). In rats given 0.1 mg. testosterone-4-C<sup>14</sup> of high specific activity, concentrations of radioactivity were localized in ventral prostates (399). *Pseudomonas testosteroni*, a micro-organism able to adapt to the utilization of certain steroids as its sole carbon source, can oxidize androstene-3,17-dione, 1-androstene-3,17-dione, testosterone, and 4-androstene-3,17-dione to 1,4-androstadiene-3,17-dione (400). Gonadectomy and injections of estradiol-17 $\beta$

and testosterone in rats indicate that the male hormone promotes the ability of the liver to reduce the sidechain of cortisone while the female hormone has an inhibitory effect (401).

The administration of testosterone propionate at 25 to 100 mg. per day for up to 56 days to partially regulated depancreatized-orchietomized dogs produced a constant increase in body weight accompanied by a retention of nitrogen and a decrease in glucose excretion. The nitrogen effect gradually "wore off" after three weeks of androgen treatment, but the effect on body weight and glucose excretion continued for the duration of treatment. On cessation of injections the nitrogen excretion increased but the glucose excretion gradually returned to the basal level. The blood glucose and nonprotein nitrogen changed in parallel with the urinary constituents (402). The simultaneous administration of 2.5 mg. per day testosterone abolished the increased protein catabolism induced by 10  $\mu$ g. *dl*-thyroxine in hyperthyroid, castrated rats and produced an effect on urinary nitrogen excretion identical with that in non-thyroxine-treated rats (403).

Large doses of testosterone block the urinary electrolyte effects of deoxycorticosterone (DCA) acetate in adrenalectomized rats. The androgen reversed the Na/K response to DCA by simultaneously blocking Na retention and K loss (404). Androstanazole (17 $\beta$ -hydroxy-17 $\alpha$ -methylandrosterone-[3,2-C] pyrazole) was found to be 30 times more anabolic than methyltestosterone when evaluated after oral administration in orchietomized male rats (405). Pullets receiving testosterone propionate demonstrated a significant increase in red blood cell count and hematocrit and a decrease in spleen weight (406). A-norprogesterone antagonized testosterone-induced accessory sex organ hypertrophy in immature orchietomized rats and comb-growth stimulation in the chicken (407).

Androgens exhibited an increase in specific activity when administered orally in oil solution instead of aqueous suspensions in rats (408). Testosterone is readily absorbed in rectal suppositories in the human male (409).

Dehydrogenation of dehydroepiandrosterone, a 3 $\beta$ -hydroxysteroid, is histochemically demonstrable in all types of steroid-producing cells in the adrenal gland, ovary, and testis of the rat (410). Finally, it is apparent that nature has endowed the reproductive tract of the male and the female with many close relationships. This is beautifully seen in the cytologic structures of the sex cells, their biochemistry, and the wondrous way in which each sex is adjusted to the other (411, 412).

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## PERIPHERAL CIRCULATION<sup>1</sup>

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Readers interested in the literature on peripheral circulation of the year covered by this review will find themselves both blessed and swamped with a bumper crop of reviews (10, 11, 101, 112, 124, 175, 251, 304), symposia (54, 60, 136, 296 to 301), compilations (66, 308), and books (204, 332). The main body of the current survey considers in a general way the passive and active determinants of peripheral resistance. For information concerning specific vascular beds, methods, etc., the reader is referred to the literature indexed in the last half of the review beginning on page 312. Contributions from physiology's precocious progeny, rheology and biophysics, are noted superficially in the first section; those from our more mature relatives in pharmacology, anatomy, and biochemistry have been neglected because of space limitations.

### THE PASSIVE DETERMINANTS OF PERIPHERAL HEMODYNAMICS

Progress in this field depends most urgently on an integration of knowledge of the biological and physical sciences; either operating without the support of the other will lead to sterile and erroneous ends.

#### THE BLOOD

Studies have been described in which the dynamic properties of blood have been divorced from those of its physiological container by causing it to flow in small glass tubes. A plot of pressure-flow relationships under these conditions (143) exhibits, at low values, a curve with convexity toward the pressure axis, but which soon becomes linear, as the values increase. The rate of flow that occurs in arterioles is well in the range depicted by the linear portion of this curve. This indicates that non-linear relationships between these parameters seen in perfused vascular beds must result from the physical characteristics of the vessel wall rather than those of the blood. This generalization has also been applied to the process of critical closure since blood in glass tubes does not have a yield value (143). Extensive theoretical consideration of data obtained, in which a microphotographic technique was used to determine the degree of thinning of the red cell concentra-

<sup>1</sup> Review of the literature was closed in June 1960. The reviewer realizes the impossibility of complete coverage and regrets omissions of significant contributions. He can only hope that the virtue of their accomplishment may be adequately rewarding to the contributors, and apologize to the real losers, those who are left uninformed.

<sup>2</sup> The reviewer is devotedly grateful to Dr. Ruth McVaugh, Miss Jeannie Pierce, and Mrs. Patricia Goulet for their superior and indispensable contributions to the preparation of this manuscript.

tion in the peripheral sheath of blood in a glass tube (25), has led to the conclusion that the formation of a marginal zone of reduced red cell concentration is quantitatively inadequate to account for the reduction in apparent viscosity of blood at increased flow rates. The electrical conductivity of blood increases 2 to 5 per cent as the velocity of blood flow rises from 0 to 15 cm. per sec. (224).

The flurry of excitement about the cell separation process has subsided with the judgment that it probably has little to do with renal hemodynamics (222). It must be remembered, however, that hemoconcentration does play an as yet undetermined role in increasing the viscosity, and therefore the resistance to flow, of blood through the efferent arteriole (156). Some of the increase in resistance to flow observed when the kidney perfusate is cooled to 20°C. is attributed to an increase in viscosity of blood (185), and it is estimated that a decrease in temperature of the extremities from 29 to 11°C. causes a 50 per cent increase in blood viscosity (79).

In contrast to the apparently small part normally played by the physical characteristics of blood, the total blood volume is an important determinant in hemodynamics, particularly in venous return. Some insight into the role played by volume receptors in the maintenance of a constant blood volume is being developed [Welt (321)]. There is evidence (24) that there are discrete receptors at the thyrocarotid junction which, when subjected to a fall in pulse pressure, negotiate an increase in aldosterone secretion by the adrenal cortex and that there are receptors in the atria and pulmonary vessels which, when stimulated by normal transmural pressure, send afferent impulses up the vagus that inhibit aldosterone production. A decrease in central blood volume produced either by hemorrhage or by positive pressure breathing (20) results in a many-fold increase in antidiuretic hormone release. Right atrial distention with a balloon decreases aldosterone secretion even after hypophysectomy but not after vagotomy (12). No modification of the hyperaldosteronuria or sodium retention produced by constriction of the thoracic inferior vena cava is seen after chronic vagotomy and carotid sinus denervation (67).

Various mechanisms are considered to be responsible for shifts in hematocrit values. Hemoconcentration produced by epinephrine has been attributed to splenic contraction (13), that in exercise to loss of water into the active muscle (70), and that in hypothermia to "loculation" of plasma-rich blood in parts of the vascular bed (327). Further studies on shifts in hematocrit values have been reported (102, 125, 239, 240).

#### THE VESSEL

*Structure of the vessel wall.*—A major consideration given to the structure of the blood vessel in current physiological literature is its relation to the development of hypertension, and this relationship will be reviewed under that heading. Failure to find pores of adequate size between the endothelial cells in the small vessels (88) and the demonstration of vesicles within these

cells form a basis for questioning the current physiological concept of the mechanism of movement of water across the capillary wall and for considering the possibility of the involvement of pinocytosis in this process.

The problem of the structural development of new blood vessels in areas deprived of an adequate blood supply is now coming into focus, but the determinants of the growth of such vessels are still in the realm of mysticism. Weibel (317) has had this process under observation in the lung and has reported his findings in an impressive picture story. No more can be done by way of analysis of the mechanism responsible for the reconstruction process than to say that it may be governed by either humoral or pressure factors, but physiological studies (65, 325) have demonstrated its quantitative significance. While the normal bronchial circulation receives only between 1 and 2 per cent of the output of the left ventricle, the reconstructed system, seven months after ligation of the left pulmonary artery in the dog, received between 15 and 30 per cent of the cardiac output. Likewise, in four patients with evidence of pulmonary disease the circulation through the bronchial arterial system received between 3 and 12 per cent of the cardiac output. Development of new vessels has been observed under special conditions in the myocardium (29, 249) and in thrombi (303).

*Static distensibility of the vessel wall.*—In a discussion that maintains close contact with biological reality, Peterson (246) analyzes the physical characteristics of blood vessel wall in terms useful to the biologist. He considers the physical characteristics which determine the amount of strain (distention) that will be produced by a given stress (increase in pressure). From a derivation of LaPlace's law he notes that the usual practice of omitting the factor of wall thickness is faulty. The "stiffness" of the vessel wall is influenced by all of its structural components: (a) elastic and collagen connective tissue, (b) water and electrolyte content, and (c) vascular smooth muscle tension. Contrary to Burton's concept (79) that the elastic modulus of a wall is decreased by increasing smooth muscle tone, Peterson, Jensen & Parnell (247) have demonstrated that there may be as much as a tenfold increase in stiffness of the vessel wall when its smooth muscle is made to contract. Studies of pulse wave velocity (178) and pulmonary artery distensibility (241) also suggest that the coefficient of elasticity of the vessel wall increases with an increase in smooth muscle tone. Critical opening pressure has been used as a measure of the smooth muscle component of tension in the vessel wall (109). A diminished bradycardic response to the infusion of norepinephrine (92) and an exaggerated fall in pulse pressure in response to amyl nitrite (91) are good indications of a rigid vascular system. Distensibility of a carotid segment changes after whole-body irradiation (288). Distensibility of the vascular bed of the lung has been widely studied and will be discussed under pulmonary circulation.

*Dynamic distensibility of the vessel wall.*—The physical characteristics of the blood vessel wall have been analyzed by study of its dynamic responses. Peterson *et al.* (247) have used this principle in a technically refined form to



describe the visco-elastic characteristics of the aorta of the dog. Pressure changes throughout the cardiac cycle were recorded by a high-fidelity capacitance manometer, and the associated displacement of the artery wall was monitored by means of a differential transformer capable of measuring movements of 0.5 micron. Outputs of these instruments, reflecting respectively the stress and strain of the artery wall, were fed into tape recorders. These tapes were subsequently administered to computers which described various parameters of the artery wall. The strain (change in circumference) an artery undergoes with a normal pulse varies from 1 to 4 per cent of its circumference. This strain depends primarily on the elastic characteristics of the vessel; however, it is influenced also, to a minor degree, by viscous characteristics (a blood vessel is stiffer if pressure is changed rapidly than if it is changed slowly). The force necessary to overcome the inertia of the artery wall under physiological conditions is negligible. The normal variations in the mechanical moduli (elastic and viscous properties) of the thoracic aorta are small, presumably because of the minimal content of smooth muscle in this section of the arterial tree.

MacDonald & Taylor (213) consider the arterial circulation as being in a steady state of oscillation, driven by the frequency of the heart beat. This contrasts with the Windkessel concept which treats the pulse as a transient phenomenon. From recordings of central and femoral pulses and with the aid of an analogue computer, Stacy & Giles (291) characterize the mechanical properties of the arterial wall in terms which may have some practical diagnostic value (if diagnosticians were available who could operate this system). Other studies (85) analyzing arterial pressure pulse contours permit an assessment of the relative magnitude of the forward and reflected components of the waves. The velocity of pressure pulse transmission has been studied critically intravascularly (178) and extravascularly in polyethylene tubing (306). In the latter, pressure pulse transmission is so rapid that negligible error is introduced when it is used to monitor intravascular phenomena.

Lawton (183) approaches the problem of the physical characteristics of vessel walls using a different technique and philosophy. From determinations of the natural frequency of strips of blood vessel wall, he calculates an "elastomeric parameter" which defines the ratio of the unstretched length of a macromolecular chain to the maximum extension which it can undergo. Since Lawton uses strips of vessel cut parallel to the long axis of the vessel and since the vascular smooth muscle and most of the connective tissue fibers run in a circumferential direction, one wonders what "macromolecular chain" is studied. Further problems arise in assigning biological significance to this study since there was "drying and deterioration of the specimen over the long course of the experiment". It would appear that this application of a physical tool to a biological problem is as appropriate as the use of a turbojet engine to launch the Kitty Hawk. While indicting the use of powerful physical tools on a weak biological base, Malcolm's speculations (199,

200) should be noted behind their thin veil of mathmanship and experimental observations. He proposes that Korotkov's sounds have origin in the collapse of cavitation (gas formation in low-pressure areas in the brachial artery). In his second monograph, which only by its title, *Blood Pressure Sounds II*, seems appropriate for this review, he wanders from the physical basis for this sound, through synapse transmission by "photosynthetic inductance", to the cause of melanotic sarcoma without evidence that his tongue is in his cheek.

Before leaving the review of the physical characteristics of the vascular system, it should be noted that in their analysis of the relationship between stress and strain in the artery wall, Peterson *et al.* (247) demonstrate that the arterial wall does not undergo active contraction synchronous with the cardiac cycle.

#### THE ACTIVE DETERMINANTS IN PERIPHERAL HEMODYNAMICS—VASCULAR SMOOTH MUSCLE

Superficial consideration of the first two sections of the review suggests that the subject matter to be dealt with could be sharply divided into (a) that dealing with the nonsmooth muscle elements of the vessel wall and (b) that related to the properties of vascular smooth muscle. Such rigid simplification is, however, incompatible with the nature of biological science. Complications exist on the one hand because the physical properties of smooth muscle contribute to the passive (elastic, viscous, and inertial) characteristics of the vessel wall, and on the other because the total tension in the vessel wall is the sum of that of its nonsmooth muscle and smooth muscle (i.e., passive and active) components. This complexity makes it very difficult to deal with pure smooth muscle contributions. Nevertheless, Folkow (94) has described a means of expressing the relative magnitude of this active component. As a reference point he measures vascular resistance with its smooth muscle completely relaxed (e.g., by a supramaximal concentration of acetylcholine). The vessel wall in this condition has an irreducible minimum of tension or of influence on resistance to blood flow. It is possible and useful to express the quantity of active tone as a ratio of the actual resistance to the resistance at this baseline level. In making such a description of the quantity of active tone, it is necessary to specify and to maintain constant the distending pressure at which the resistance measurements are made, since the viscoelastic properties of smooth muscle differ from those of vascular connective tissue. For this reason, as the distending pressure (and therefore the total tension in the vessel wall) increases, the relative contributions of smooth muscle and of nonsmooth muscle components change. Additional complications exist in the use of this ratio as a measure of active tension of vascular smooth muscle. For the following reasons neither the magnitude nor the effect of the distending pressure can be perfectly described: (a) the distending pressure differs greatly over a segment of resistance vessel, diminishing in a variable and undefinable fashion as the capillary bed is approached,

and (b) the total tension in the wall opposing a given distending pressure will be less in small resistance vessels than in large ones (LaPlace's law). Because of these complexities the ratio of existing resistance to the minimal resistance must not be interpreted as an accurate measure of active vascular smooth muscle tension. However, this does seem to be the best available indirect index of it.

Current literature invites a comment about the terms used to describe peripheral resistance. This force opposing the movement of blood is always measured as a pressure drop per unit flow; however, it is expressed either directly in the parameters in which it was measured (pressure drop and unit of flow) or in a conversion of these values to a force measurement expressed in dynes sec. per cm.<sup>6</sup> In the literature on systemic circulation the former expression predominates, whereas in that on pulmonary circulation the latter is more commonly used. A plea for uniformity of expression of resistance values seems in order; and, since the direct expression in terms of the units used in its measurement gives a concrete picture of what this value actually means and since the expression in terms of a physical force is not ordinarily used for further derivations, the former seems preferable.

#### INTRINSIC PROPERTIES OF VASCULAR SMOOTH MUSCLE

The basic machinery involved in the performance of vascular smooth muscle has received less attention than that of any other tissue of comparable significance. Investigations have dealt more with the effects of environmental changes on this performance than with the nature of the contractile machinery itself.

The many factors, both intra- and extraluminal, which influence the performance of this tissue make it difficult to study its intrinsic properties in a controlled fashion in an *in vivo* environment. Haddy (134) has levelled two major objections at information derived from studies on isolated vessels: (a) results obtained with large vessels such as have been used for *in vitro* studies cannot reasonably be inferred to apply to small muscular arterioles which are the chief determinants of vascular resistance, and (b) isolated tissue studies suffer "from the inadequacies of an unphysiologic perfusion fluid." The reviewer is soon to describe a technique which permits direct recording of tension development by arteriolar smooth muscle. With regard to the second objection, it is true that differences should be expected between the response of such an isolated muscle and its behavior in the intact animal; however, since the basic contractile requirements and properties of arteriolar smooth muscle can be analyzed much more critically in the isolated bath where the environment can be completely controlled and the mechanical responses measured directly, a definition and analysis of these differences between *in vitro* and *in vivo* responses should lead to the detection of significant *in vivo* influences. Encouragement of the idea that the bath of physiologic salt solution does not reduce all vascular smooth muscle to an identical performance has been evident in two studies. Mallov (201) noted a qualita-

tive difference between the responses of vascular smooth muscle from normotensive and those from hypertensive rats to increases in sodium concentration in the bath. Setnikar & Rouasi (282) found that control aortic strips contracted in response to nicotine, while those taken from rabbits pretreated with reserpine did not. This retention of an *in vivo* characteristic, also seen in other types of smooth muscle (128), argues that the isolated system may be used to analyze altered characteristics of the muscle in the intact animal.

The oxygen requirement for the contractile process in vascular smooth muscle has not been defined. Evidence of an altered vascular performance occurring during ischemia or following the administration of cyanide to a perfused organ (165) cannot be interpreted as indicating that these factors have a direct effect on vascular smooth muscle. It is likely that in hypoxia the surrounding parenchyma contributes metabolites which act on the vascular smooth muscle.

There are equally barren areas in the field of electrical activity and electrolyte shifts accompanying the responses of vascular smooth muscle. Raab (255) indicates that there are two laboratory groups who failed to observe electrical activity associated with the mechanical response of vascular smooth muscle to epinephrine; one of these notes electrical activity following response to electrical stimulation. Evidence has been presented (144) which corroborates this suggestion of a basic difference between the response of smooth muscle to electrical stimulation and to stimulation with epinephrine; the former was accompanied by shifts in electrolyte composition while the latter showed no such associated electrolyte change.

Folkow (94) has re-emphasized the fact that following denervation there remains a large component of myogenic tone. This maintained tension in the absence of neurogenic influence is most evident *in vivo* (55, 110) but is also seen in some isolated arterial preparations (330). The determinants of this nonneurogenic vascular tone have not been defined. There is no satisfactory information about the relationship of this continuous state of active tension to the rhythmic contraction of vascular smooth muscle that is seen both *in vivo* (3, 326) and *in vitro* (236, 237). The question whether myogenic propagation of an excitatory process occurs in vascular smooth muscle needs further clarification. Burnstock & Prosser (44) present evidence which suggests that no such propagation occurs. However, there seems to be no way to account for the rhythmic activity in noninnervated vascular smooth muscle without intercellular synchronization through myogenic propagation. Fulton (105) has observed the propagation of the mechanical response in cocaineized vessels of the terminal vascular bed; and in his electron microscope pictures of vascular smooth muscle, Fawcett (88) has shown blunt cellular projections extending through the intercellular matrix, possibly forming a structural basis for a propagated excitatory process. The propagated process described by Hilton (152) is peculiar in that it is a relaxation and in that although it persists following acute denervation, it is lost when the nerve fiber degenerates.

*Vascular responsiveness.*—Vascular responsiveness is a valuable concept in peripheral circulation. Both in common usage and in its exact sense, it describes the magnitude of response of vascular smooth muscle that results from a given stimulus. It is measured with various degrees of purity. (a) A pressor agent may be administered to an intact animal and the magnitude of the pressure rise used as an index of vascular responsiveness (e.g., 133, 151, 235). The weakness of this approach is that an observed change in "responsiveness" may be caused by altered cardiac performance rather than by change in vascular smooth muscle. (b) The change in peripheral resistance of an intact animal or vascular bed may be measured (e.g., 55, 96, 171). Here, however, the magnitude of a recorded response can be expected to vary with the thickness of the wall and the degree of tonic contraction of the smooth muscle at the time the test stimulus is applied (96). Neither of these variables can be readily evaluated. Two additional factors complicate the interpretation of a change in vascular resistance as a direct measure of the change that occurs in the smooth muscle. Resistance is a function of the fourth power of the length of the smooth muscle. There is no way of knowing how much of the muscular contraction is manifested as a decrease in length, and how much as an increase in tension. The *in vivo* contraction is neither purely isometric nor isotonic, and the relative changes in length and tension will vary even as the tension in the wall changes during a given response. (c) The most direct method for evaluating the contractile response is to make a recording of its shortening or tension development (72, 330). This process usually requires that the muscle be studied in an isolated bath where both physical and chemical environments differ so from those in the body that predictions cannot be made that any *in vitro* influence will have the same effect *in vivo*. The technique described by Peterson *et al.* (247) may afford a satisfactory but complicated solution to the problem of measuring vascular responsiveness.

#### RESPONSE TO TENSION INCREASE—AUTOREGULATION

One of the most interesting and certainly most controversial subjects currently appearing in the literature on peripheral circulation is the problem of autoregulation. The basic question whether the vascular smooth muscle in the resistance vessels contracts in response to an increased transmural pressure has not been adequately answered. The problem is fascinating because of the diversity of results obtained, the interesting mechanism that must be involved, and the implications regarding the role played by this mechanism in the control of blood flow through various organs and in hypertension.

The kidney is the vascular bed most used for these studies. Langston *et al.* (182) saw no increase in resistance as the perfusion pressure was raised from 60 to 300 mm. Hg. Hinshaw's group (153 to 156) found that total renal vascular resistance did increase with increased perfusion pressure, and they present extensive evidence supporting their hypothesis that the increase is

due to an increase in renal tissue pressure which tends to obliterate intrarenal veins. Other investigations (280, 283) seem to furnish convincing evidence that the resistance vessels do contract in response to an increase in transmural pressure.

The observation has been confirmed (26, 290) [and refuted (46)] that cerebral blood flow tends to remain constant with perfusion pressures above 60 mm. Hg, demonstrating that vasoconstriction and increase in resistance must occur at higher pressures. If the "closure mechanism" (214) of the internal carotid and vertebral arteries observed following occlusion of the jugular veins is real, it may be attributable to a similar response to an increase in vascular pressure. Johnson (165) attributes a "venous-arteriolar response" in the mesenteric vascular bed to an increase in transmural pressure in the arteriole. This response can be eliminated by the administration of cyanide, ischemia, or papaverine. Small arterial segments of the extremities also constrict in response to an increase in intraluminal pressure (166). Blair *et al.* (35) interpret their studies as indicating that vasoconstriction in response to an increase in vascular transmural pressure occurs in the human forearm.

An interesting feature of autoregulation is that it requires that the increase in the transmural pressure causes the vessel to constrict to a circumference smaller than it was before the increase in pressure was applied. This eliminates the possibility that the process is maintained by lengthening of the over-all circumference. In order to account for the maintenance of such a myogenic response, it is necessary to visualize an elastic element in series with the smooth muscle cells which, by imposing increased tension on the individual cells, causes these to shorten so that the over-all circumference is less than it was before the tension in the wall was increased.

The spasm response to the mechanical stimulation of puncture of a vein (41) and the positive chronotropic response to stretching of cardiac pacemaker cells (242) may both be brought about by mechanisms closely related to that of autoregulation. It is interesting to speculate about the role that such processes may play in arterial hypertension.

#### TEMPERATURE

A compilation of extensive but ancient literature includes many references concerning the effect of hypothermia on the circulatory system (66). Cold has been shown to be both a relaxer (38) and a contractor (237) of isolated vascular smooth muscle. In the intact vascular bed, a decrease in temperature is usually reported to cause an increase in vascular resistance (79, 109, 185). This is attributed both to a direct vasoconstrictor action of cold and to an increase in blood viscosity. Cold-induced vasodilatation (79) has opened an interesting area for speculation as to the mechanism involved; both metabolite accumulation and critical opening and closing of arteriovenous anastomoses associated with changes of viscosity in the blood seem worth considering. Adrenalectomy results in failure of cold-induced cutaneous vasocon-

striction (329). This is corrected with cortisone treatment. Pressor responses to intravenous epinephrine persist at body temperatures of 22°C. in the rabbit (287).

#### ELECTROLYTES

The roles of sodium and potassium in the performance of vascular smooth muscle have continued to be favorite subjects for observation and speculation. Sodium and sodium-retaining hormones command major respect in experimental and clinical hypertension (5, 56, 98, 101, 114, 122, 137, 139, 140, 304, 328). A good case has been made for the concept that the responsiveness of vascular tissue bears an inverse relation to the transmembrane sodium gradient (103, 104, 255), but this is based on indirect evidence. Measurements of the intracellular sodium concentration in vessel wall have not been made because of difficulties occasioned by the large amount of bound sodium in the wall (73, 145). Indirect support for this gradient theory is seen, however, in studies in which the total body intracellular sodium was found to increase over threefold with the development of hypertension in response to deoxycorticosterone treatment (51). Some evidence suggests that there is a similar change in vascular smooth muscle (174).

In contrast to the above thesis, popular among the hypertensionologists, Dodd & Daniel (73) feel that the sodium gradient per se is not responsible for vascular contractility. Furthermore, Yamabayashi & Hamilton (330) observe an increase in response to both epinephrine and norepinephrine when the sodium chloride concentration in the bath is increased to 1.2 times that in Krebs solution. They argue that this is a specific potentiating effect of the sodium cation rather than a nonspecific increase in osmolarity, since a similar increase in the latter produced by glucose failed to give a comparable increase in response. Mallov (201) observes a similar potentiation of the response of normal vascular smooth muscle to epinephrine when the sodium concentration is elevated. However, he attributes this to the increase in osmolarity since a similar potentiation is produced when the osmolarity is increased with sucrose. Using vascular resistance as an index of smooth muscle activity, Haddy (134) notes that an increase in sodium chloride concentration is attended by a decrease in this parameter. Read *et al.* (258) contend that such a vasodilatation is caused by a nonspecific osmolar action. The direct action of hypertonic solutions in producing a decrease in vascular resistance appears to be well documented (172, 208). When contrasted with the somewhat opposite effects observed in isolated vessels (201, 330), this action suggests that there is a basic difference between the smooth muscle of large vessels, used in the latter studies, and that of the small resistance vessels. A qualitative reversal in the effects of a hypertonic solution on the isolated vessel was noted when animals were made hypertensive with DOCA (201). In the intact animal or the perfused vascular bed, the vasodilator effect of hypertonic solutions is complicated by various reflexes that may be initiated (172) and by possible red cell agglutination (208, 279).



It seems plausible to explain the long-term action of chlorothiazide, observed by Conway & Lauwers (56), in reducing total peripheral resistance as the result of a change in the content or distribution of sodium in the blood vessel wall. A determination of how much of this decrease in resistance is due to a diminution in vascular smooth muscle responsiveness and how much to a lessening of the water content of the vessel wall should be a valuable contribution to the understanding of the mechanism of the increase in vascular resistance in hypertension.

The schism between the results of studies on isolated blood vessels (38, 73) and on perfused vascular beds (83, 276), (or probably more pertinently between those studying large and small vessels), is seen also in studies on the effect of potassium. In the isolated large vessel, responsiveness seems to be a direct function of potassium concentration while in the perfused system, an increase in potassium causes a decrease in responsiveness to both norepinephrine and acetylcholine. Differences of opinion exist concerning the role of intracellular potassium in the effects of this cation (23, 73). It has been conjectured that when epinephrine causes smooth muscle excitation, it is accompanied by a potassium efflux (81), contrary to previously cited observations (144). It is also suggested that some of the cardiovascular effects of histamine may be attributed to the increase in plasma potassium concentration which it effects (141).

Haddy (134) has shown that, while an increase in magnesium causes a fall in small vessel resistance, as is the case with most other cations, calcium causes a persistent increase in both large and small vessel resistance but decreases the responsiveness to norepinephrine. An increase in hydrogen-ion concentration was shown to have the usual vasodilator effect in an isolated perfused vessel (237), but to produce an increase in responsiveness in the isolated perfused vascular bed (36).

The need in this complicated and contradictory electrolyte story is for an evolution of the goal of this research from its current one, which is largely descriptive of the effects of electrolyte shifts in various experimental situations, to one of analysis of the mechanism by which a specific electrolyte produces its effect.

#### HUMORAL CONTROL

*Catecholamines.*—Although circulating catecholamines probably play a minor role in total peripheral resistance compared with unknown determinants of vascular smooth muscle tone in the plasma, they occupy a major position in the literature (see also p. 314). Two symposia (298, 299), one review (11), and one book (204) have been devoted to catecholamines, with varying levels of pertinence to the subject of this review.

Lindgren, Rosén & Uvnäs (188) have determined threshold infusion rates for vasoconstrictor and vasodilator responses to catecholamines in skeletal muscle, skin, and intestine. This group (189) notes that activation of the sympathetic vasodilator outflow system in the cat probably does not release

enough catecholamines from the adrenal medulla to have a significant vascular effect. Burn & Rand (43) indicate that the postganglionic sympathetic nerve endings have a mechanism for taking up circulating norepinephrine released by the adrenal medulla; such an accumulation of this catecholamine reduces the threshold for neurogenic vasoconstriction. The increase in circulating epinephrine and norepinephrine during hemorrhage has been measured (116, 204, 310); the catecholamine content in the peripheral blood has been evaluated during anesthesia, struggle, and fracture (311), during a cold pressor test (169), and during diffusion respiration (219); and both epinephrine and norepinephrine content in the venous blood of patients with Reynaud's disease have been found to be elevated (243), while levels of these amines in the hypertensive patient are normal (204).

The mechanism of action of catecholamines on vascular smooth muscle has received some attention (106), but little progress has been made beyond a careful definition of the problems. The question of whether the vasodilator effect of epinephrine is due to a direct action of this agent on the contractile machinery or whether it is secondary to metabolic effects is still debated (81, 106, 221). Complexity is added to the problem of the contractile machinery by the fact that the shortening of vascular smooth muscle by catecholamines occurs in two separable stages (38).

Further observations have been made on the development of denervation sensitivity. From the observation that there was no increase in sensitivity to low concentrations of epinephrine while the constrictor effect in response to higher concentrations was greatly increased, Cooper, Willman & Hertzman (58) suggest that the vasoconstriction that occurs in response to low concentrations occurs in the precapillary sphincters which may never be innervated. He further interprets these observations as indicating that physiological concentrations of endogenous epinephrine in the blood do not contribute to therapeutic failure following clinical lumbar sympathectomy. The potentiation of an epinephrine response following treatment with ganglionic blocking compounds (84) may be related to the mechanism of denervation sensitivity; however, this seems unlikely because the potentiation occurs as soon as the agent is administered (133). This potentiation of an epinephrine response by ganglionic blocking may be fairly specific since the pressor response to angiotensin is not similarly potentiated (133). Evidence from several sources (133, 151, 235) demonstrates that ganglionic blocking agents are capable of potentiating pressor responses by a peripheral vascular sensitization. The frequently made observation that epinephrine oxidation is catalyzed by heavy metals appears in the literature once more (284). The arterial pressure fall during a prolonged infusion of norepinephrine results from a decrease in cardiac output (181).

*5-Hydroxytryptamine.*—Second in the list of attention-getting humoral agents is 5-hydroxytryptamine. The most popular target has been the pulmonary vascular bed where it causes constriction of both the pre- and post-capillary vessels (8) and probably also has a direct effect on pulmonary cap-

illary permeability (168). Although it has been maintained (285) that these effects are produced by concentrations of 5-hydroxytryptamine found in the plasma of patients with carcinoid tumors, evidence is presented that the pulmonary artery pressure in this disease is not much elevated (50). 5-Hydroxytryptamine has been found to be the most potent constrictor of the umbilical artery (236). An interesting observation that small doses of 5-hydroxytryptamine diminish the responses to epinephrine and norepinephrine, whereas large doses of this agent potentiate the response to catecholamines, has not been satisfactorily explained (273). 5-Hydroxytryptamine has been implicated along with epinephrine and norepinephrine as playing a role in regulating blood flow through the skin (333), and its role in burns (164) and other vascular phenomena (59) has been considered.

*Other humoral agents.*—Evidence presented by Haddy (135) indicates that histamine in low concentrations causes an increase in small vein pressure by arteriolar dilatation alone, whereas in high concentrations this pressure rise is augmented by venous constriction. He believes that the elevation in capillary hydrostatic pressure is sufficient to account for histamine edema. Aviado (8) obtained quite different results with histamine in the pulmonary vascular bed in which he saw only precapillary constriction. Tachyphylaxis to angiotensin II has been observed in the isolated blood-perfused kidney (19), and in studies on the isolated aorta strip (121) it was noted that the response to this agent diminishes with prolonged periods in the bath, while there is no such diminution in the aorta's response to epinephrine. This loss of responsiveness to angiotensin does not require previous exposure of the aorta to angiotensin. Langford (181) suggests that tachyphylaxis to renin may be caused by occupation of angiotensin receptor sites by an inactive metabolite of angiotensinogen. Bradykinin is a physiologically occurring vasodilator agent (21). The callicrein system has been dealt with (160, 316) but no evidence of its physiologic or pathologic significance has developed. Secretin and pancreozyne both cause vasodilatation in the pancreas, an action which is not inhibited by either atropine or mepyramine (158). A basis for concern about vasoactive materials released by blood as it passes through polyethylene tubing has been described (271).

#### NEUROGENIC CONTROL

Two excellent symposia (296, 300) and three reviews (124, 175, 251) reflect an increasing interest in this aspect of the control of peripheral circulation and have done much to develop a productive intercourse between neuro- and cardiovascular physiologists.

*Afferent systems.*—The major sensing devices whose activity regulates the neurogenic control of vascular smooth muscle are stretch receptors in the wall of the blood containers. Peterson (245, 246) describes the operation of these with a "black box" system having a clarity that would satisfy a first grader and a perceptiveness that would challenge a sophisticated biophysicist. "Negative feedback" is still a useful teaching concept in these systems,

and his thesis, that the "pressure" receptors are not really stimulated by pressure directly but rather by the stretching of the vessel wall as a consequence of intravascular pressure, gives insight into the important role that wall "stiffness" must play in their operation. Other useful reviews of the pressure receptor system appeared (191, 228, 229).

Numerous interesting facets have been added to our large base of information about the operation of stretch receptors in the carotid sinus area. It has been noted (76), contrary to previous reports, that in the rabbit this pressure receptor reflex is active from the first day of life. Since mean arterial pressure rises from an average of around 40 mm. Hg during the first week of life to 65 mm.Hg by the end of the second week, it seems that the threshold of mean arterial pressure required to excite the baroreceptors increases over this period. Another example that may reflect the "stiffness" of the vessel wall as a determinant of the sensitivity of these stretch receptors is the demonstration that in patients with evidence of sclerotic vascular changes, there is a definite depression of the bradycardic response associated with norepinephrine infusion (91, 92). There may also be a diminished reflex responsiveness in hypertension (150). Pressure receptors with a depressor influence in the adjacent thyrocarotid arterial junction have been found in the dog (107). In hypothermia the pressor response to carotid artery occlusion is exaggerated at first but disappears completely at 22°C. (287). Rushmer (264) has noted that the carotid sinus reflex can still be elicited during periods when the central nervous system controlling exercise is dominating cardiovascular dynamics. The carotid sinus system probably plays a major role in compensating for positive gravitational stress (34).

Further contributions have been made regarding the efferent pathways through which the carotid sinus afferents may mediate their effects. No increase in epinephrine-like substance in the plasma was observed in the dog following common carotid occlusion (204). Indirect evidence is presented which can be interpreted as indicating that stimulation of pressure receptors causes a pulmonary vasodilatation (77). This observation is in accord with the thesis presented in a new translation (315) of an old monograph by Wasserman which maintains that acute pulmonary edema is of reflex origin and can be counteracted by pressure on the carotid sinus. An increase in pressure receptor activity appears to cause a bronchiolar constriction (186).

Several intrathoracic sensing systems having an effect on vascular dynamics have been explored. Coleridge & Kidd (53), in a neat study monitoring action potentials in single afferent fiber preparations in the vagosympathetic trunk, demonstrate the presence of stretch receptors in the two branches of the pulmonary artery but not in the main pulmonary artery trunk. Salisbury, Cross & Rieben (266) describe the reflex effects of left ventricular distention. With circulation maintained by an extracorporeal lung-pump, inflation of a balloon in the left ventricle to give a diastolic pressure that exceeded 10 mm.Hg caused bradycardia, a decrease in total peripheral resistance, and venous dilatation (a large decrease in the extra-

corporeal blood volume). Evidence continues to accumulate (242) which makes the reviewer wonder why it takes the Bainbridge reflex so long to die. There may be receptors in the lung which respond to emboli (167) and to hypertonic solutions (172) (or are these the same thing?). The subject of volume receptors (12, 20, 24, 67, 321) has been dealt with in the section on blood. Peterson (246) suggests that a volume receptor is merely a pressure receptor that happens to find itself in the wall of a distensible tube.

Whitteridge (324) describes many interesting vascular reflexes that are initiated from receptor sites outside of the cardiovascular system itself. Thus the blush on the bride may merely be due to the situation in which she has been too preoccupied to empty her bladder. Hemodynamic studies during bladder distention in patients with low cervical transverse myelitis show increases in pulmonary and systemic vascular resistance (2). Stretch receptors which cause a pressor reflex and whose afferents travel up the vagus are found in the esophagus and in the cardia and pylorus of the stomach (61). Support has been found for the presence of pressor afferents in the vagus (170). The existence of a depressor reflex originating in the pancreatic arteries (272) probably sets a record for brevity (37, 149); its presence could not be confirmed experimentally by controlled perfusion of this vascular bed. Stimulation of the afferent stump of the splanchnic nerve in the dog and cat usually causes a pressor response, in the rabbit a depressor response (97). Evidence of this species difference was also seen in that the rabbit demonstrated a depressor response to acoustic stimuli while the other two species failed to produce a pressure change (294). Vasodilatation has been observed in the perfused hindlimb of the dog after stimulation of sympathetic afferents in the contralateral chain (28).

Various parameters of the response to chemoreceptor activity have been reviewed (175) and investigated (90, 108, 176, 193, 219, 322). MacCanon (197) notes that diastolic arterial pressure and pulse rate fall and the cold pressor response is potentiated when normal males are shifted from room air to pure oxygen.

*Parts of the central nervous system involved in peripheral vascular control.—*

A theme is currently evolving that there are in the central nervous system discrete structural or functional sites for the control of blood flow through certain organs and for the integration of such physiological responses as exercise and love (300). The work horse of this whole operation is still the medullary vasomotor center, and numerous observations have been made characterizing details of its function [see Oberholzer (232)]. In the rabbit this center ceases to function when cooled to 22°C. (287). Electrical activity recorded from this area in the unanesthetized cat during progressive anoxia (90) has shown a pattern of changes which end in electrical silence accompanied by a fall in arterial pressure. It is likely (193) that postasphyxial vasodilatation results from the persistent activation of a dilator center which is masked during asphyxia by the hyperactivity of a constrictor center. This of course would mean that the activity of the former outlasts that of the lat-

ter. As more concentrated solutions of ammonium chloride were poured over the floor of the fourth ventricle (195), the initial pressor response changed to a depressor response even when the pH shift of the ammonium chloride was compensated, and it was suggested that the stimulating and inhibiting effects of this agent act at different sites. *d*-Tubocurarine inhibits the medullary vasomotor center but has no effect on responses arising from electrical stimulation of the hypothalamus (244). Reserpine does not inhibit the medullary vasomotor center (162). Pressure in an isolated venous segment falls when the subject faints (41) and rises when he does an arithmetic problem (210), which suggests that the patient in congestive failure should be frightened but should not be subjected to mathematics.

Getting to centers above the medullary level of command, Glasser (115) has found an excitatory center in the lower pons which drives the cardio-accelerator and vasomotor neurons of the medulla. There is an inhibitory center in the mid pons which balances this drive. Several studies appeared which differentiate the cardiovascular activities of different regions of the hypothalamus. Areas discharging to the heart tend to be somewhat more ventral than those discharging to the vascular system (205). Folkow (95) has identified a sympathetic inhibitory center in the hypothalamus which when stimulated causes a hypotension which is unaffected by atropine. Distinct from this area, and ventral to it, is situated the cholinergic vasodilator system to skeletal muscle. The "energy effector system" of Uvnäs, Lindgren & Rosén (188, 189, 305) now seems well established; originating in the motor cortex it travels down through the hypothalamus, collicular area, and ventrolateral medulla where it remains quite separate from the vasomotor centers. Stimulation of one motor cortex causes bilateral vasodilatation (223). This seems to be the same system whose effects on the heart have been studied by Rushmer, Smith and their associates (263, 264, 289). Both are probably required for the cardiovascular response to exercise. Thus, when a lesion is placed in the system, the dog's legs may run but his heart won't know it (289). Pappenheimer (238) indicates that there may be an area in the brain specifically for control of circulation to the kidney.

*Efferent systems.*—To be consistent in this "reflex arc-type" treatment of the neurogenic control of the vascular system, the organization of the current section will progress on out the efferent limb of the arc, treating first the ganglia, then the efferent constrictor and vasodilator nerve activity, the nerve endings, and finally some definition will be made of the activities of the vascular system not under neurogenic influence.

The role of ganglia is studied almost exclusively by noting the effect of ganglionic blocking drugs. Aviado (9) describes the hemodynamic effects of these agents, and many publications have appeared indicating the limitation of use of these agents in an evaluation of the activity of the efferent limb of neurogenic control of blood vessels. Two main problems seem to exist: (a) these agents have actions other than that of ganglionic blockade (132, 133, 171, 282); and (b) ganglionic blockade may not be complete (157, 327).

The rhythmic changes in the size of the vessels in the finger are not related to changes in arterial blood pressure (42), which suggests that sympathetic outflow controlling these variations is different from that in control of arterial pressure. Rhythmic activity of preganglionic sympathetic fibers has been observed both before and after treatment with reserpine (162). Variations in sympathetic outflow with anesthesia are particularly prominent in studies on the neurogenic control of the kidney (15, 238).

Many experiments deal with the vasodilator response mediated over the sympathetic system. The acoustico-depressor reaction observed in the rabbit is considered to be due entirely to an elimination of sympathetic constrictor tone (294). Heat dilatation occurring in the hand is likewise due entirely to the release of sympathetic constrictor tone, while that occurring in the forearm is caused additionally by active dilatation mediated to some extent by bradykinin (21). The same pattern applies to the vasodilatation occurring in the hand and forearm following insulin hypoglycemia (4). The vasodilatation mediated over the "energy effector system" is thought to be blocked by atropine (188, 189, 305). A vasodilator response observed in the dog tongue after the administration of horse serum to which the dog has been sensitized involves, in some mysterious fashion, a cholinergic system (1). Postasphyxial vasodilatation may involve histaminergic nerves (193). Beck & Sakuma (28, 265) have described further studies on a histaminergic vasodilator system in the dog hindleg. This finding will be revolutionary if, as the authors reported earlier, the histaminergic system plays a role in active dilatation in response to an elevation in systemic pressure.

Studies on adrenergic nerve endings indicate that they are capable of taking up circulating norepinephrine (43) and that their norepinephrine stores may be released by stimulation with nicotine (220). The complexity of the receptor system for adrenergic substances in the smooth muscle cell has been emphasized in reviews by Green & Kepchar (124) and Furchgott (106). The differences among these receptors on smooth muscles of different vessels (72, 123), together with their complex pharmacological behavior (250), has made the problem an attractive one for arm-chair philosophy.

Finally it should be of value to re-emphasize that although the nervous system plays a major role in the regulation of blood flow and blood pressure, in its absence there still persists a high level of vascular smooth muscle tone. This relationship is dramatically demonstrated by Conway (55) in the dog hindleg; removal of smooth muscle activity by an intra-arterial infusion of a supramaximal dose of acetylcholine led to as much as a twenty-fold increase in flow over that seen in the denervated leg. Other evidence of the autonomy of vascular smooth muscle has appeared (58, 105).

#### ACTIVE AND REACTIVE HYPEREMIA

There is a tendency for vasodilatation to occur whenever the blood supply is reduced to a level lower than that necessary to provide an adequate oxygen supply (reactive hyperemia) or whenever tissue activity is increased so that



the oxygen demand exceeds the amount supplied by the blood (active hyperemia). It is probably reasonable to assume that dilatation in the two cases is caused by the same mechanism. The speculation is safe since neither mechanism is really known. Reviews by Korner (175) and Green & Kepchar (124) have dealt extensively with this subject, and interesting experimental evidence has appeared.

Hilton (152) has made the most extensive study of this problem. He confirms an older observation that, when a skeletal muscle is active, the artery supplying it dilates. He demonstrates that this dilatation has its origin in the active muscle and is propagated up the artery at a velocity of about 10 cm. per sec. He argues that this propagation speed is slow for nerve conduction, yet too fast to be due to the transport of a dilator substance in the limbs. Furthermore, the propagated dilator response is not affected when the adventitia is stripped from the vessel, nor is it abolished by posterior root section, sympathectomy, atropine, phentolamine, or local cocaineization. The response will not occur when the animal is hypotensive, suggesting that the smooth muscle of its walls will not relax in response to the stimulus unless it is stretched initially. Many similarities between this large artery dilatation and the postcontraction hyperemia of skeletal muscles are described in support of the proposition that the two are caused by the same mechanism. Another example of propagated vascular dilatation following infrared irradiation of the skin has been described (63). When the proximal part of the forearm is irradiated, there is a concurrent vasodilatation in the distal part as far as 12 cm. from the nearest irradiated skin. Other investigators (62, 331) have been impressed by the parallelism between the magnitude of reactive hyperemia and the arteriovenous oxygen difference. Although one of these groups (62) concludes that the reactive hyperemia is due to a lack of adequate oxygen supply to the vascular smooth muscle cell itself, it is questionable whether this conclusion is justified in view of the many other changes that occur in the environment of these cells when the skeletal muscle is anoxic. A good case can be made for oxygen supply being either a direct or indirect determinant of coronary blood flow (27, 33).

#### PARALLEL COUPLED RESISTANCES

Folkow (93) lists the following determinants of the distribution of blood flow through the various organs or "parallel coupled circuits": (a) differential input activity over afferent systems, (b) critical integration in vascular control centers, (c) varying degrees of innervation of vascular smooth muscle. To these it would seem reasonable to add the determinant (d) variable sensitivity of smooth muscle to neurogenic, humoral, metabolic, and pressure influences. Several studies should be singled out for their emphasis on differential flow through the various parallel vascular beds (9, 124, 189, 270, 305). The pulmonary vasculature is not a parallel circuit and will be considered in a separate section.

## BRAIN CIRCULATION

Methods of measurement of cerebral circulation and its regulation have been considered in some breadth in two publications (267, 290). Adrenergic nerves or substances apparently have little or no effect on cerebral vascular resistance (31, 170, 212). Changes in arterial pressure at levels above 60 mm. Hg probably have little effect on blood flow (26, 290); however, a conflicting result has been reported (46). The most effective agent for increasing cerebral blood flow is hypercapnia (187, 290). Hypoxia causes a lesser increase. With a new tracer technique for evaluating regional blood flow, it was shown that increased physiological activity in the optic lobe of the brain is accompanied by an increase in blood flow to this area (290). Since the brain requires between 10 and 20 per cent of the body's oxygen supply at rest, it is surprising that dogs can survive (but poorly) for months after ligation of both common carotid and vertebral arteries (40). Minimal success has been recorded in an isolated perfused dog-brain preparation (234). Little has been done to solve the mysteries of the blood-brain barrier (68, 206) and the formation of cerebrospinal fluid (30). Cerebral blood flow is normal in patients with mild congestive heart failure, moderately reduced in those with severe heart failure, and greatly reduced in those patients who demonstrate symptoms of confusion (80, 281).

## CORONARY CIRCULATION

Consideration has been given to the structural (29, 253, 254) and functional (202, 249) aspects of the microcirculation of the coronary bed. Blood flow through different parts of the heart and through the hearts of different species is a direct function of the amount of work done by the specific myocardium involved (147). The myocardium operates on high oxygen extraction rather than high blood flow (33). Neurogenic influences can have a significant effect on coronary blood flow (274, 275, 302). In an extensive study from Japan (142), the authors argue that any direct vasodilator effect of the sympathomimetic amine operates through a metabolic mechanism rather than through a specific receptor for vascular smooth muscle relaxation. Roentgenologic visualization of the coronary flow (322) has been used to study the actions of many physiological and pharmacologic agents. The vasodilator actions of serotonin (211), histamine (202), hypertonic solutions (258), and a new pharmacologic agent (48, 113) are impressive. Blood flow through the myocardium is maintained after small hemorrhages (270), but after a large hemorrhage it decreases and is a major determinant in the deterioration of the animal. Major systemic arteriovenous fistuli (117) cause an increase in coronary flow. The strength of contraction of the ventricle is a function of the coronary blood flow rate and not of the perfusion pressure (27).

## KIDNEY CIRCULATION

The sympathetic supply to the kidney has a potent vasoconstrictor action (180) which tends to be activated by anesthesia (15, 238). This probably

accounts for the observation that only 27 per cent of the blood flow of the inferior vena cava is contributed by the renal veins (218). Catecholamines (217, 223), angiotensin (19), and hypothermia (185) are potent renal vasoconstrictors, whereas a moderate elevation in potassium concentration (276) tends to decrease renal vascular resistance. The increase in lymph flow and sodium concentrations observed following an increase in renal venous pressure is worth thinking about in connection with the pathogenesis of congestive failure (184). Of all of the vascular beds, that of the kidney is most drastically restricted in hemorrhage (270). Split renal function tests indicate that the difference in blood flow between the two kidneys tends to be much greater in the hypertensive (14) than in the normal (161). A fall in arterial pressure to the kidney may be the direct stimulus for the release of renin from juxtaglomerular cells (304). Further studies relating the kidney to the pathogenesis of hypertension will be listed in the section on that disease. Autoregulation in the kidney has already been discussed.

#### OTHER VASCULAR BEDS

Studies of blood flow through the liver suffer from the indirectness of the methods for its measurement (17, 47, 75, 218, 292). Evidence continues to accumulate indicating that a normally circulated liver protects against shock (277).

The vessels supplying skeletal muscle are the most sensitive in the body to the vasodilator effect of epinephrine (189). This observation may be related to the vasodilator effect of insulin hypoglycemia. However, some of this latter dilatation is attributable to a neurogenic cholinergic action (4). Active and reactive hyperemia and autoregulation in skeletal muscle have already been discussed. Skeletal muscle vasodilatation is incriminated in the collapse of the compensatory mechanisms in hemorrhagic shock (270).

Vascular smooth muscle in skin has a relatively high threshold for its constrictor response to catecholamines (189). Both bradykinin (4) and 5-hydroxytryptamine (333) are implicated in the physiological control of circulation through the skin. Propagation of vasodilatation produced by infrared radiation is presumably neurogenic (63).

Splanchnic blood flow studies indicate that the decreasing metabolic gradient at lower levels of the gastrointestinal tract is accompanied by parallel decreases in blood flow (111). Neither epinephrine (189) nor anesthesia (86) seems to alter intestinal vascular resistance. However, since administration of the former agent is accompanied by an elevation in arterial pressure it is argued that there must be an increase in active tone in the vessel wall in order to prevent a distentional decrease in vascular resistance. Perhaps this is further evidence for intestinal autoregulation (165, 166). Sapirstein, Sapirstein & Bredemeyer (270) observe that splanchnic blood flow is not disproportionately curtailed in hemorrhage despite the "teleological attractiveness [of such a curtailment] which has withstood observations to the contrary for more than 30 years". Detailed consideration is given to the

regulation of splenic vasculature (123) and its blood content and composition (13, 256).

Blood flow through the adrenal gland is maintained or increased in the rat with hemorrhage (270) while in the dog it is decreased (116). A surprisingly large blood flow is found through vasa vasorum (269), and this flow is not altered by whole-body irradiation (288). In the submaxillary gland (82) a histamine vasodilatation in response to the administration of *d*-tubocurarine was observed. However, the vasodilatation seen in the pancreas in response to secretin and pancreozyne is not mediated through either histamine or acetylcholine (158). A sympathetic constrictor action on blood vessels in bone marrow is demonstrated (319).

#### SERIES-COUPLED DIVISIONS OF THE VASCULAR TREE

Folkow (93, 94) has re-emphasized the value of considering the vascular tree in sections progressing from the aorta to the vena cava; various levels differ in their function and in their response to different types of controlling influences. This survey will merely list current experimental evidence demonstrating these differences.

Structural differences have been observed between the type of lesion that develops in response to deoxycorticosterone in the large artery and in the arteriole, and this difference is dependent on the activity of the pituitary gland (139). Qualitative and quantitative differences between large and medium-sized arteries in their responses to pharmacological agents (72) are reported. Haddy *et al.* (83, 134, 135, 276) observed differences between the responses of vascular elements at various levels of the vascular tree, one of the most striking differences being in the response to histamine, which causes dilatation of the arterioles and constriction of the veins. It is suggested that the precapillary vessels are the ones most sensitive to epinephrine (58). Emphasis is placed on the independence of the resistance vessels and those determining finger volume (42); the changes in finger volume may reflect activity of the pre- and postcapillary vessels concerned with the exchange of fluid across the capillary wall.

Differences in control among resistance, nutrient, and capacity vessels are found. The increase in blood flow accompanying activation of sympathetic vasodilator systems is not accompanied by an increase in clearance of radioactive sodium from the muscle (305); this may result from the opening of nonnutrient vessels, thus establishing an oxygen-conserving system. Differences exist between the control of the inflow and outflow vessels of the spleen, which determine its volume (123). The increase in renal vascular resistance produced by norepinephrine is accompanied by an increase in renal volume (217). Folkow (93), using a system which permits differentiation of the responses of pre- and postcapillary vessels and those of capacity vessels, has shown that the capacity vessels are more sensitive to nerve stimulation than are the resistance vessels, and that the constrictor fibers have a greater influence on precapillary than on postcapillary resistance. Epineph-

rine dilates resistance vessels at the same time that it constricts capacity vessels; norepinephrine constricts both, and angiotensin constricts predominantly the resistance vessels. Before leaving this subject which is so directly involved with hydrostatic pressures in the capillaries, it should be pointed out that direct measurements of capillary pressure have been repeated (198), their results confirming the original values obtained by Landis.

### PULMONARY CIRCULATION

The lesser circuit has received major attention in the literature covered by this report. For this reason and because this system cannot logically fall into either the parallel or the series coupled resistances, it merits a separate section.

There can be no doubt that the pulmonary vascular bed has a nerve supply which is capable of producing a large increase in pulmonary vascular resistance (10). It is also certain that this vasoconstrictor system is activated by anoxia (8), as well as by other afferent influences (2, 77, 167, 315); however, its role in the maintenance of normal pulmonary vascular resistance is yet to be defined. Although there is agreement that low oxygen tension acting systematically causes a reflex pulmonary vascular constriction, its effect on vascular smooth muscle in the lung is not clearly established. There is support for both direct dilator (8, 10, 175) and direct constrictor (69, 203) effects. The local effects of high carbon dioxide are probably constrictor (7, 203).

5-Hydroxytryptamine still holds its position of supremacy as a pulmonary vascular constrictor (8, 168, 230, 262, 285); it may cause a diversion of blood through arteriovenous anastomoses (230), and it has been shown to be a constrictor of both the arterial and venous side of the pulmonary vascular tree (8). Norepinephrine causes constriction and an increase in stiffness of the pulmonary artery (241), and adrenergic materials may play some role along the 5-hydroxytryptamine in reflex vasoconstriction (168). Both 100 per cent oxygen and acetylcholine are moderately effective in lowering pulmonary vascular resistance in pulmonary hypertension (207, 286, 295). Procaine (120) and theophylline ethylenediamine (22) are also dilators. Vasoconstrictor actions of histamine (8, 293) and angiotensin (268) are ill defined. There is a slight elevation of pulmonary artery pressure in exercise (248).

The fall in pulmonary vascular resistance that occurs in one lung when the contralateral pulmonary artery is occluded with a balloon occurs equally well in patients with chronic pulmonary hypertension (39, 49) and in normals. Studies such as these which relate the resistance of the pulmonary vascular bed to its flow and distending pressure must recognize the complicating determinants of critical closure, intra-alveolar pressure, and neurogenic reflexes (8, 49, 192, 194). Parallel problems arise in attempting to ascribe a change in pulmonary vascular resistance to a change in active smooth muscle tone (261). An isolated, submerged, gas-free lobe has been used (194) to study the effects of variations in arteriovenous and transmural

pressure gradients on this resistance; the system behaved analogously to a tube of lesser elasticity within a tube of larger diameter and greater elasticity. A straight-line relationship is revealed between changes in radius and distending pressure when simultaneous records of artery size and pressure are made over a physiological pressure range (241).

The blood volume in the lungs has been determined by various techniques and under different conditions (173, 190, 209, 278, 314, 320). From a diffusion study it has been estimated that the volume of the pulmonary parenchymal tissue in man is approximately 600 ml. (45). The pulmonary membrane has been found to be of little value as an artificial kidney (64). Guyton & Lindsey (130) calculate that the movement of fluid across the alveolar membrane normally follows expected gradients of hydrostatic and osmotic pressures.

### ABNORMALITIES OF THE CARDIOVASCULAR SYSTEM

Because of the enormity of the subject, atherosclerosis, the major abnormality of the cardiovascular system, will not be dealt with in this review.

### HYPERTENSION

Reviews, symposia, and books (101, 136, 159, 304) continue to keep the reader up to date with the confusion in this subject. Based on rather flimsy evidence, the classical concept that there is no increase in cardiac output, but that the elevation in arterial pressure is due to an increase in total peripheral resistance has been maintained. The pot at the end of the colorful rainbow of research is therefore supposed to have in it information about the cause of the increase in total peripheral resistance. Its possible content may be worth itemizing.

(a) Impressive evidence has been presented indicating that structural changes occur in the vessels in hypertension, restricting the degree of vasodilatation that can occur (55, 110, 226, 312). The problem in using this structural restriction as an explanation for the rise in pressure is that there is much evidence that a change in pressure itself is capable of producing change in the structure of blood vessels. For instance, chronic administration of epinephrine results in a change in the connective tissue of the vessel wall (196), and pressure change is recognized as being an important factor in vessel growth (317).

(b) Although it is commonly accepted that vascular responsiveness, in its loose sense, increases in hypertension (55, 101), it is not clear whether this change is ascribable to a real change in vascular smooth muscle or merely to a change in vessel wall thickness or to a decrease in the baseline circumference of the vessels. The observation that there is a qualitative difference between the performances of vascular smooth muscle from normal and hypertensive animals (201) does much to keep open the possibility that the primary change may be due to a real change intrinsic in the smooth muscle. In the present state of our information, it is probably not profitable to specu-

late whether the vast accumulation of literature regarding the influence of salt and endocrine factors argues for structural changes or for changes in vascular smooth muscle reactivity (5, 32, 51, 74, 98, 99, 100, 103, 104, 122, 137 to 140, 174, 255).

(c) The kidney still receives extensive support as a producer of pressor material (118, 131, 132, 146, 268). There has been an impressive extension of the literature relating the juxtaglomerular apparatus to the formation of renin (57, 59, 71, 148, 227, 304). Probably the most spectacular evidence relating the kidney to the cause of hypertension has been the clinical finding of the high incidence of unilateral kidney abnormalities and of cures resulting from nephrectomies (14, 52, 78). Few of these studies argue directly for a renal pressor material as a cause of hypertension; the pressure elevation may equally well have been caused by a structural change in the vessel or by a change in vascular smooth muscle reactivity. The following findings conflict with the hypothesis of a renal pressor mechanism of hypertension: (i) renal vascular changes have been dissociated from the development of hypertension (6, 323); (ii) renal autoexplants protect against renoprival hypertension (226); and (iii) it has been demonstrated that grafting a normal kidney on a hypertensive rat will cause its arterial pressure to fall (119). These observations are interpreted as indicating that the kidney has a specific "metabolic orcretory" function which opposes the pressor mechanism of hypertension. Again this says nothing about the characteristic of the vascular change that is responsible for the hypertension. Tachyphylaxis to renin and angiotensin has been observed (19, 181) and may be an argument against these substances being the cause of hypertension.

(d) Little support has been evident for a neurogenic mechanism in hypertension (150). Circulating catecholamine content is normal (204).

#### SHOCK

Most of the studies designed to explore the mechanism of hemorrhagic and traumatic shock have developed negative or equivocal results. The vaso-depressor material (VDM) is still inactive (163, 260), and the germ theory has not fared much better (18, 216). Studies exploring the roles of callicrein (316), 5-hydroxytryptamine (215), and corticosteroids (309) have been non-contributory. In the face of a very large increase in circulating catecholamines (204, 310, 313), there is a decrease in responsiveness to these agents (313). This may have some connection with a vasodilator material elaborated in the anoxic intestine (277). A many-fold increase in lactic dehydrogenase has been found in the plasma, paralleling biological evidences of deterioration following severe hemorrhage (307). A most valuable approach to the analysis of the problems in hemorrhagic shock has been a study of the altered distribution of cardiac output that occurs in moderate and severe hemorrhage (270).

One review (112) and several studies (16, 126, 233) have dealt with the mechanism of shock produced by bacterial endotoxins. Pooling of blood in



the hepatosplanchnic bed plays a major role in the hemodynamic effects of this material.

### METHODS

Measurement of blood flow is the subject of one book (231) and of one symposium (297). Electromagnetic flowmeters (89, 259) and temperature-dependent flowmeters (75, 79, 218, 225), some with surprising potentialities, have been described elsewhere. Specific techniques designed for measuring flow through the following vascular beds are either new or of particular interest or both: bronchial artery (65), pulmonary capillary bed (45), renal (217), hepatic (17), coronary (322), and cerebral (187, 267, 290).

In connection with pressure measurements, various sections of *Intra Vascular Catheterization* (332) are worth noting. A technique for measuring dynamic pressures in the microvasculature is admirably developed (257). Indirect methods for blood pressure recordings are noted (252, 318), and a classical and convincing analysis of the basis for Korotkov's sounds is presented (177).

Other devices that every laboratory worker in peripheral circulation would like to have are: a catheter-type  $pO_2$  electrode for continuous measurement of blood oxygen tension (179); a very simple and dependable device for continuous chronic injections (87); equipment for continuous measurement of cardiac output, equipped with analog computer (129); and a differential transformer capable of measuring blood vessel size to the nearest .5 micron (247). Finally, since extracorporeal circulation is becoming a favorite indoor sport among cardiovascular physiologists, the following studies using this technique should be of interest (127, 171, 258, 266, 271).

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## HEART

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### INTRODUCTION

The publications cited in this review appeared between July 1, 1959 and June 30, 1960; a small number of publications appearing before the first date are mentioned either because of special interest or as necessary reference material. The review is limited in scope, in part because of the interests and range of competence of the authors and in part because of limitations of space. In general it considers the electrical and mechanical activity of heart muscle; hemodynamics, electrocardiography, and clinical cardiology are not included.

### ELECTRICAL ACTIVITY

#### TRANSMEMBRANE POTENTIALS

Few if any basic studies on the transmembrane potentials of cardiac muscle have been published this year. Hoffman & Cranefield (1) have reviewed much of the published material and included some unpublished data. Coraboeuf (2) presented an extensive review of his own studies and some of the results of others. Several other short review articles have appeared (3, 4). Déléze (5) studied the effect of lowering temperature on the transmembrane action potential and concluded that the major part of the decrease in resting potential during cooling results from slowing of the sodium pump. Chang (6) demonstrated regenerative repolarization of cooled Purkinje fibers in response to weak inward current pulses. Crill (7) studied the effect of membrane current on the transmembrane potential of heart cells in the cultured chick embryo; the time constant was found to be 35 msec. and results of polarization were the same at widely different electrode separations. Vaughan Williams (8, 9) studied the relationships between atrial transmembrane potentials and afterpotentials and contraction; the results are considered in the section on mechanical activity. Paes de Carvalho (10) has presented evidence, based on studies of the transmembrane action potential, for the existence of specialized fibers in rabbit atria. Monophasic action potentials recorded simultaneously through suction and intracellular microelectrodes have been compared (11); the suction electrode appears to give an accurate indication of the voltage-time course of the membrane potential during all but the upstroke of the action potential. However, spurious afterpotentials in the suction electrode record may be created by movement. Surawicz *et al.* (12) used simultaneous records of the electrocardiogram, the monophasic action potential, and the transmembrane action potential to study the effects of potassium and calcium deficiency.

There have been a number of reports on the effects of drugs on transmembrane action potentials. Matsumura & Takaori carried out a series of studies of the effects of common cardiac drugs on ventricular muscle fibers from rabbit hearts (13) and also on fibers of the atrium and sinoatrial node (14). Of particular interest are the results obtained with digitalis glycosides, quinidine, and procaine amide. Matsuda *et al.* (15; see also 78) have reported a detailed study of the effects of aconitine on dog ventricle. Local application of this agent causes oscillatory afterpotentials which increase in amplitude and, on attaining the threshold potential, elicit repetitive firing. This action is prevented by an excess of either calcium or magnesium. Aconitine increases the rate of diastolic depolarization of sinoatrial pacemaker fibers (15). The actions of tetraethylpyrophosphate hydrobromide on the sinoatrial node and atrium of rabbit heart are similar to those of acetylcholine and can be reversed by pyridine aldoxime methiodide (16).

Metabolic activity has been related to transmembrane potentials in several papers. MacFarlane (17) compared the effect of a number of enzymatic inhibitors on frog ventricle with that of excess potassium and suggests that active ion transport is important in production of the plateau of the action potential. Carlos de Mello (18) studied the effects of dinitrophenol and azide on sinoatrial and atrial fibers in rabbit heart and also the reversal of dinitrophenol effects by ATP. Gargouil and co-workers recorded transmembrane potentials from rat ventricle at different ages (19) and studied the effects of thyroid hormone on electrical activity of this tissue (20). Dower (21) reported on transmembrane action potentials recorded from guinea-pig ventricle and demonstrated the extent to which these records are distorted by electrical activity occurring elsewhere in the ventricle. This paper should be compared with an earlier report (22). Vaughan Williams (23) also described the relationships between surface electrograms and transmembrane action potentials. Noda (24) showed that part of the transmembrane potential is not abolished by eliminating the potassium concentration gradient across the membrane, and suggests that the spike and plateau of the action potential have different bases. The recovery of the membrane resting potential in normal Ringer, following KCl depolarization, has a much slower time-course at 4°C. than at 20°C. (25). Addition of ATP (0.1 mgm. per ml.) to the medium partially reverses KCl depolarization of frog ventricle strips (26).

*The syncytial nature of cardiac muscle.*—Electronmicroscopic observations have demonstrated the continuity of the intercalated disk membrane with the inner layer of the sarcolemma of Purkinje fibers (27), ventricular fibers (28 to 30), and atrial fibers (31). Anatomical studies of the embryonic development of heart muscle have strengthened the opinion that the intercalated disk is a complete transverse subdivision of the fiber (32) and its contents. Recently a number of investigators have assumed that the double membrane of the intercalated disk acts as an effective membranous separation of cardiac muscle into electrically discrete cells and that the transmission of excitation from one cell to another depends on some form of junctional transmission. Sperelakis (33) has measured the electrical resistance between

two microelectrodes when only one is inserted in a fiber and when both are inserted in fibers at varying separations. Also, the effect of perfusion of hearts with several hypertonic solutions has been recorded through surface and intracellular electrodes (34). The main points of evidence in support of junctional transmission at intercalated disks are as follows: when two microelectrodes are intracellular, interelectrode resistance is double that seen when one is extracellular; hypertonic perfusion causes asynchronous activity in surface electrograms and transmembrane potential recordings; during hypertonic perfusion, records of transmembrane potential may show slow depolarizations preceding the action potential upstroke or appearing in the absence of action potentials; finally, one-way conduction may occur in ventricular muscle. In a related study Hoshiko and associates (35) compared the change in specific resistance of cardiac, smooth, and skeletal muscle caused by perfusion with sucrose solution. The greater increase in specific resistance of cardiac and smooth muscle has been interpreted as evidence for the non-syncretic nature of these tissues.

A rigorous evaluation of the resistance measurements made with microelectrodes is possible only if all the passive electrical properties of the excitable fibers are known, as well as the linear separation of the electrode tips. Areas of inexcitable membrane may be produced even in single nerve fibers (36); such areas may show graded depolarization with or without an all-or-nothing response. The production of one-way conduction should be evaluated in terms of the experiments of Drury (37), Schmitt & Erlanger (38), and others. Finally, it has not yet been shown that the inner layer of the sarcolemma is the seat of the major part of the electrical resistance of the excitable membrane. However, even if the intercalated disks are only a low impedance barrier across the myocardial fiber, they will be the site of delayed conduction and block whenever the safety factor of propagation is reduced by a sufficient amount. This does not mean, however, that the normal myocardium is not a functional syncytium.

#### EXCITABILITY

Van Dam (39) has written a monograph (with English summary) presenting a large number of experiments carried out on isolated papillary muscles and intact canine hearts. One major conclusion, in general agreement with results of earlier studies (40, 41), is that the "dip" in the strength-interval curve results from anodal stimulation. Also, studies of refractoriness at different depths in the free wall of the ventricle showed that the innermost layers generally have the longest duration of refractoriness [see also (49)]. During total occlusion of a coronary artery branch, there was almost total loss of excitability in the involved area while partial occlusion caused an increase in both anodal and cathodal thresholds (39). Brooks *et al.* (42) also studied changes in excitability in ischemic heart muscle and report a decrease in duration of both the monophasic action potential and refractory period and an increase in threshold and in latency of response. Ueda (43) has recorded from injured ventricular fibers through intracellular microelec-

trodes. In the area surrounding an injury caused by crushing or heating, there is a decrease in the magnitude of the transmembrane resting potential and action potential. Interestingly, anodal polarization fails to increase action potential amplitude in this area. In the injured area thresholds are elevated and the absolute refractory period is prolonged. Membrane resistance, measured with a bridge circuit, is decreased by injury. In another study of excitability, ligation of the anterior descending coronary artery caused fibrillation thresholds to decrease to 20 per cent of normal (44). Walker (45) studied the effects of anodal and cathodal polarization and found that the former induces coupled beats originating during the T wave while the latter causes coupling during the P-R interval. Strength-duration curves obtained from cooled dog hearts by monopolar anodal or cathodal stimulation (46) show a greater effect of temperature on anodal excitability and a greater change in thresholds for stimuli of short duration. Yasutomi (47) reported studies of the excitability of atrial muscle, and Stein (48) studied the effects of reserpine on the excitability of turtle heart. Recovery of excitability at different depths in the ventricular myocardium has been related to the polarity of the epicardial T wave (49); flat or negative T waves appeared when the end of the effective refractory period of epicardial muscle followed completion of recovery in deeper layers, and positive T waves were associated with recovery of the epicardium in advance of deeper layers. Engelking & Bienroth (50) studied the effect of the intramural temperature gradient on the electrocardiogram. Tada (51) determined the effect of several concentrations of acetylcholine on conduction velocity of atrial muscle (51). Pillat & Heistracher demonstrated a supernormal conduction velocity and a decreased latency for extrasystoles occurring late in the refractory period in cat papillary muscle (52).

#### RHYTHM

There have been few studies of the basic mechanisms underlying pacemaker activity in cardiac muscle during the past year. A good deal of information is summarized in the review by Coraboeuf (2) and in another short review by Hoffman (53). Some additional studies are included in the section on Ions.

Milton related pacemaker activity in isolated rabbit atria to acetyl-CoA-kinase activity (54) and studied the relationship between choline acetylase activity and the maintenance of membrane potential (55). Evidence has been presented for an action of acetylcholine on infranodal pacemakers of rat heart (56). Swaine & Waud (57) have shown that certain synthetic steroid bases decrease heart rate and cause some peripheral block of the actions of norepinephrine. A careful study has demonstrated that thyroid hormone influences the rate of pacemakers located not only in the sinoatrial and atrio-ventricular nodes but also in the ventricles of cat and rat hearts (58). An effective method has been devised for evaluation of the effect of depressants on infranodal pacemaker activity in the intact animal (59). Waud (60) showed that epinephrine and norepinephrine have equal effects on the rate of

the heart-lung preparation and that their potency is not influenced by reserpine. Glenn (61) described a method for remote stimulation of the heart by radiofrequency transmission, and Kahn (62) described a ventricular pacemaker which is triggered by electrical activity of the atria. The heart rate of turtles has been found unrelated to body size (63). Maintenance of rhythmicity in frog heart has again been related to emissions from radioactive elements (64).

#### ARRHYTHMIAS

Kossman (65) reviewed the importance of a knowledge of the activity in pacemaker fibers in understanding the generation and treatment of clinical arrhythmias. Reviews have appeared on the interpretation of complex arrhythmias (66) and the differentiation of supraventricular and ventricular tachycardias (67).

*Atrial arrhythmias.*—Burn (68, 69) has recently summarized his views on those factors which predispose to atrial or ventricular fibrillation and on the nature of these arrhythmias. The conclusions are based on a large number of studies including the effects of temperature and ions (70), anoxia (71, 72), autonomic influences (69), and other agents. In general, he finds that a decrease in the duration of the transmembrane action potential increases the likelihood of fibrillation and that prolongation of the action potential makes this arrhythmia less likely. His views should be compared with those expressed by Moe & Abildskov (73), DiPalma (74), and Katz & Pick (75). Also, they must be considered in relation to the findings of Moore & Swain (76) on production of ventricular fibrillation by an agent which has as its primary effect the prolongation of the action potential. Lyons and co-workers (77) studied atrial flutter, fibrillation, and tachycardia and summarized the factors which tend to maintain or arrest atrial flutter. They present evidence for the possibility of either a circus movement or a focal discharge in this arrhythmia and emphasize the importance of the interplay between mass of muscle, duration of refractoriness, and conduction velocity in maintenance of arrhythmias.

Moe & Abildskov (73) conducted a series of experiments which demonstrate that atrial fibrillation is a self-sustaining arrhythmia and is independent of focal discharge. This is in contrast to conditions maintaining atrial flutter (77). Schmidt (78; see also 15) studied, in the dog, the effects of aconitine on Purkinje fibers and papillary muscle fibers with microelectrodes. An early increase in the rate of slow diastolic depolarization in Purkinje fibers is followed by a progressively lengthening positive afterpotential (at a level of 60 mv. membrane potential), from which the fibrillation potentials arise. A reduction in sodium concentration ( $\frac{1}{2}$ ) or a fourfold increase in calcium concentration produces a temporary repolarization to the original diastolic membrane potential with cessation of fibrillation. No effect of aconitine on the inactivation characteristics of the sodium carrying system was observed, nor were changes in membrane resistance (from time-course of electrotonus) detected. These studies re-emphasize the importance of local

differences in excitability in production and maintenance of fibrillatory arrhythmias. Holland (79) has carried out additional experiments related to the theory (80) that atrial fibrillation is initiated whenever transmembrane fluxes of  $\text{Na}^+$  and  $\text{K}^+$  exceed critical values. Replacement of  $\text{Cl}^-$  by  $\text{SCN}^-$ ,  $\text{I}^-$ ,  $\text{Br}^-$ , or  $\text{NO}_3^-$  has been found to inhibit fibrillation in isolated rabbit atria in this order of effectiveness (79). All anions caused a decrease in  $\text{Cl}^-$  influx from media containing 5.9 mM  $\text{Cl}^-$  per liter; all caused some increase in  $\text{K}^+$  efflux and no demonstrable effect on  $\text{Cl}^-$  efflux. Nelson & Smith (81) showed that, during fibrillation of frog atria, activity in the sinus venosus is interrupted only occasionally by retrograde transmission from the fibrillating atria. This lack of retrograde transmission is attributed to special properties of the sinoatrial junction.

*Ventricular arrhythmias.*—An interesting pharmacologic study (76) has dealt with sensitization of dog ventricle by a substituted propiophenone (U-0882) to fibrillation induced by epinephrine, high ventricular rate, or atrial tachycardia. This agent prolongs refractoriness of atrial and ventricular muscle without changing conduction velocity in atria, ventricles, or specialized tissues. After U-0882 administration, epinephrine increases the duration of the Q-T interval. The prolonged refractoriness appears to be the direct cause of fibrillation induced by rapid ventricular or atrial rates. Protection against sensitization by U-0882 is provided by dichloroisoproterenol. In another study it has been shown that amarine differs from U-0882 in that the former compound decreases conduction velocity in addition to prolonging refractoriness (82). The relative importance of local differences in the ventricular myocardium in production of fibrillation has been the subject of several papers (73, 78). In one study, asphyxia produced by tracheal occlusion caused one-third of all hearts tested to fibrillate (83); in another, ligation of all coronary arteries caused fibrillation, but ventilation with 100 per cent nitrogen did not (84). These results imply that changes in the myocardial  $\text{pO}_2$  probably are of less importance than changes in  $\text{pCO}_2$  or extracellular potassium concentration. Ventricular fibrillation thresholds were found to drop to 20 per cent of normal after ligation of the anterior descending coronary artery and to return to 50 per cent of normal within five months (44). These changes were largely prevented by implantation in the heart of a patent branch of the internal mammary artery. Phibbs *et al.* (85) showed in studies of dogs and monkeys that hyperthermia increases and hypothermia decreases the fibrillation threshold. During hyperthermia, coronary occlusion decreases the defibrillation threshold but not below the value found in the normal heart at body temperature. Mazzella (86) has studied fibrillation in the toad and the effects thereon of temperature, potassium, and calcium. Addition of butyrate has been shown to reverse fibrillation of guinea-pig hearts induced by lack of metabolites (87).

*Hypothermia.*—Interest in the cardiac effects of hypothermia has continued (88). Lyman & Blinks (89) showed that the hearts of hibernators continue to beat at lower temperatures than do hearts of closely related non-hibernators. Several studies of the effects of temperature on metabolism and



cardiac activity have been reported (90 to 92). Cooling has a greater effect on electrical excitability and on conversion of ATP to ADP in rabbits than in ground squirrels (91). Cooling increases the tolerance of rats to hypoxia (93); treatment with potassium or with potassium and ethylenediaminetetraacetic acid decreases the incidence of ventricular fibrillation in dogs cooled to terminus (94). Effects of antiarrhythmic agents during hypothermia have been studied by several groups; quinidine was found to prevent all spontaneous fibrillation in dogs cooled to 10°C. or less in one study (95) and to have no effect on spontaneous fibrillation in another (96). In another report, antihistamines and quinidine were found to be of value in prevention of spontaneous fibrillation while procaine was of little or no help (97). The rate of rewarming after hypothermia is reported to have some bearing on the occurrence of cardiovascular collapse (98). Gerola, Feinberg & Katz (99) investigated changes in myocardial oxygen consumption and coronary flow during hypothermia. They find that, when hypothermia is induced at a fixed cardiac output, external mechanical efficiency increases. Studies of the effects of hypothermia on oxygen consumption and glycogen content of rat heart have been mentioned elsewhere [(100); see also metabolism]. Left ventricular function curves of dogs have been used to demonstrate the protective effect of hypothermia during cardiac arrest (101). O'Brien & Guest (102) also studied the functional reactivation of the hypothermic heart. Biancalana *et al.* (103) related vectorcardiographic changes during hypothermia to the onset of fibrillation.

*Drugs and other agents.*—Scherf (104) and DiPalma (74) have reviewed the current status of treatment of cardiac arrhythmias. Briggs (105) studied the inhibitory effects of several electrolyte-regulating steroids on atrial fibrillation. The mechanism of action has been interpreted in light of observed changes in potassium and sodium fluxes during this arrhythmia (79, 80); it was concluded that deoxycorticosterone, spironolactone, and quinidine act similarly and that their antifibrillatory effect stems mainly from depression of sodium entry. Matsumura & Takaori (14) studied the action of quinidine and procaine amide on the transmembrane potentials of sinoatrial fibers, and Kleinfeld *et al.* (16) showed that the action of TEPP on sinoatrial pacemakers is qualitatively similar to that of acetylcholine. Farah & Birnbaum (106) showed that amotriphene, in comparison to quinidine and procaine amide, causes a greater increase in duration of refractoriness and a similar decrease in conduction velocity. It has been shown that neither *N*-(2-diethylaminoethyl)isonicotinamide (Ambonestyl) nor quinidine causes any appreciable change in the water or ionic content of rabbit atria at concentrations which decrease rate and force of contraction (107). Tada (108) studied the antagonism between acetylcholine and physostigmine or quinidine in relation to the potassium concentration in the perfusion medium. Scherf *et al.* (109) demonstrated that small doses of calcium increase and larger doses inhibit ventricular coupling in dogs which has been induced by veratrine and 20 per cent carbon dioxide; Surawicz *et al.* (110) found that a decrease in the serum calcium level of 1 m.eq. per liter, produced by injection of ethylene-

diaminetetra-acetic acid, decreases the frequency and ventricular extrasystoles but has no effect on fibrillation or flutter. Guzman and associates (111) studied the effects of sympathomimetic agents in complete heart block during acidosis and hyperkalemia. Visioli & Botti (112) reported on the effect of local application of atropine in blocking atrial fibrillation induced by acetylcholine, and Benda *et al.* (113) reported a beneficial effect of co-carboxylase on arrhythmias resulting from infarction. The effects of sodium lactate have been compared to those of  $\text{NaHCO}_3$ ,  $\text{NaCl}$ , calcium gluconate, and glucose plus insulin (114); all increase serum pH and decrease serum potassium concentration.

#### ATRIOVENTRICULAR TRANSMISSION

Sano and co-workers (115) related transmembrane potentials recorded from the region bordering the atrioventricular node to histological examination of tissues marked through the electrode and demonstrated that action potentials having characteristics described previously (116, 117) do indeed originate from nodal fibers or from special junctional tissue adjacent to the node. Paes de Carvalho & de Almeida (118) reported a more detailed study of the relationship between transmembrane potentials of atrioventricular nodal fibers and microscopic anatomy. This work includes interesting observations on the relation between direction of spread of activity through the node and configuration of the upstroke of the local action potential. Studies of transmembrane action potentials recorded from the atrioventricular ring of the frog heart (119) show characteristics similar to those reported for the mammalian atrioventricular node (116, 117). The great similarity of the records obtained from single fibers in the atrioventricular node by a number of investigators indicates that the unusual characteristics of action potentials recorded from this region (low resting potential, slow, notched or slurred upstroke of the action potential) are not an experimental error introduced by a particular experimental technique. Records of the electrical activity of the atrioventricular node obtained through extracellular electrodes show some variation in different experiments. Sodi-Pallares and associates (120) recorded a slow potential change somewhat similar in form to that reported by Scher *et al.* (121); however, in this paper the timing of some of the nodal complexes in the cardiac cycle is at variance with the experience of most investigators. Alanis *et al.* (122) also used extracellular electrodes to record from the region of the atrioventricular node; these studies suggest, in terms of the potential identified as resulting from electrical activity of nodal fibers, that there are two sites of functional discontinuity, one between atrium and node and another between node and His bundle. An extremely detailed analysis of potentials recorded from the region of the atrioventricular node through fine extracellular electrodes has been presented for ungulate and canine hearts (123). In this study unipolar records from the atrial margin of the node reveal polyphasic deflection similar to those reported by van der Kooi *et al.* (124) while records from the node proper are similar to those of Scher and his group

(121). Variations in the form of the nodal potential have been related to the anatomy of this structure and the position of the recording electrode (123). Of considerable interest is the deflection recorded from the region of the atrioventricular node during retrograde transmission and its possible relationship to the increased nodal delay observed under this condition.

Atrioventricular transmission in young mammals has been found to differ from that in the adult (125). One of the most challenging observations is that ventricular fibrillation can be induced in young hearts by premature stimuli applied to the atrium. This finding results from a longer refractory period in ventricular muscle than in the specialized conduction system of young hearts and may be related to the observations made by Moore & Swain (76). The effect of ischemia on atrioventricular transmission has been studied (126, 127); in one case (126) the changes in transmission caused by ischemia are attributed to effects of anoxia. Jonas (128) presented a careful electrocardiographic analysis of two cases of apparent supernormality in atrioventricular conduction, and Soloff & Fewell (129) presented evidence of a supernormal phase of conduction from studies using an implanted pacemaker.

#### SPECIALIZED CONDUCTING SYSTEM

Electrograms recorded with a variety of techniques have been used to study the activity of different parts of the specialized conducting system. In isolated perfused hearts, Alanís and co-workers (130, 131) recorded unipolar electrograms from the His bundle and carried out extensive studies of the effects of a number of physiological variables such as rate, autonomic effects, and asphyxia. Conduction velocity in the His bundle was found to be 1.2 m. per sec. for both normal and retrograde activation; this agrees with the value obtained from studies of hearts *in situ* (132). These studies (131) also present evidence for block of retrograde activation between ventricular muscle and Purkinje fibers. Conduction velocity in the His bundle and in the free-running Purkinje fibers of the false tendons of the dog heart was studied under direct vision during total cardiopulmonary bypass (132); in the combined His bundle and bundle branches, the velocity was found to be 1.2-1.3 m. per sec. and in the false tendons to be 4.0 m. per sec. Sodi-Pallares *et al.* (120) recorded from the His bundle, bundle branches, and peripheral Purkinje fibers and calculated conduction velocities ranging from 2.6 to 4.5 m. per sec.; this variation results from the different speeds of conduction in different parts of the specialized conducting system (see 132). Pruitt & Essex (123) recorded unipolar electrograms from several parts of the peripheral specialized conducting system in hearts of ungulates and dogs and also carried out detailed anatomical studies of the hearts employed. Draper & Mya-tu (133) measured conduction velocity in isolated preparations of dog and goat Purkinje fibers with microelectrodes and report an average velocity of 2.5 m. per sec. Sano and associates (134) studied the problem of unidirectional block in the specialized conducting system using intracellular

microelectrodes and an isolated preparation of dog heart; block of retrograde conduction was found to be localized to a point just above the bifurcation of the common bundle, presumably because of decremental conduction at this location. This study is unique in locating block at this particular part of the Purkinje system. Electrograms recorded directly from multiple points on the specialized conducting system during cardiopulmonary bypass have been used in a comparison of the effects of hypoxia and ischemia on conduction (127).

Activation of the interventricular septum has been studied under direct vision during cardiopulmonary bypass before and after right or left bundle branch block (135). Results indicate that earliest activity is almost simultaneous on right and left sides; also, only the posterosuperior part of the right septal surface was found to be activated by excitation traveling over a path other than the right bundle branch. It was found that after right bundle branch block the Purkinje system does not participate in the endocardial spread of activity in the right ventricular wall (136). In another study, a single unipolar lead on the septum and another on the free ventricular wall were used to study septal activation under normal conditions and after bundle branch block (137); the results obtained were in general agreement with those of earlier studies. Several other reports on ventricular activation in the presence of conduction disturbances have appeared (138, 139). Some difference in the extent to which excitation from ventricular extrasystoles spreads in the Purkinje fibers of sheep and dog hearts has been noted (140).

The importance of a knowledge of the duration of refractory periods in each part of the specialized conducting system has been emphasized by several studies. Changes in the QRS complex in electrical alternans have been ascribed to effects of cycle length on refractory periods which differ in several parts of the conducting system (141). In another study, normal conduction has been restored during left bundle branch block by the pause following a ventricular extrasystole (142). Dressler (143) has presented indirect evidence that vagal activity may influence conduction and pacemaker activity in the ventricular part of the specialized conducting system. Several papers have demonstrated little mechanical asynchronism in bundle branch block or ventricular extrasystoles (144, 145); this finding is probably related to the direct effect of contraction of one ventricle on development of tension in the free wall of the other chamber.

*Anatomy.*—Atrioventricular delay and preferential conduction within the ventricle have been demonstrated in the 72-hour chick embryo before development of the His bundle (146). The specialized conducting system was described in the hearts of birds (147), rodents (148), and the Indian Wall lizard (149). Detailed anatomical studies of the atrioventricular node of rabbits (118) and ungulates (123) have been related to electrical activity of these structures. Lumb *et al.* (150) described the blood supply of the conduction system in the dog; the relationship between the arterial supply of the interventricular septum and atrioventricular conduction in the dog also

has been described (151). Carbonell (152) described the macroscopic anatomy of the conduction system and the glycogen content and enzymatic activity of its subdivisions. Attempts to demonstrate the specialized conducting system by means of intravital staining with iodine compounds (153) and with  $I^{131}$  (154) have been reported. Uhley & Rivkin (155) presented a method for visualization of the left bundle branch in human hearts. Lev (156) reviewed the anatomic basis for disturbances of rhythm and conduction. Truex *et al.* (157) completed a reconstruction of a human heart with accessory atrioventricular bundles and a clinical diagnosis of the Wolf-Parkinson-White syndrome. A pharmacologic and histologic study of the turtle heart has presented evidence for the existence of a specialized conducting system in this species (158).

### IONS

There have been a large number of studies in several species of the movement of ions in and out across the membranes of fibers from different regions of the heart; comparison of data is difficult because of variations in experimental method. One major criticism applies to a number of attempts to express in a quantitative manner "active" and "passive" components of net fluxes—the paucity or absence of accurate data on mean membrane potential and changes in concentration of the ion in the intracellular phase. Similarly, changes in "passive" movement of ions, associated with such things as altered heart rate or application of drugs, have occasionally been interpreted, without correction for changes in net driving forces, as indicating permeability changes. Because of the difficulty mentioned above, studies of ionic fluxes will be grouped on the basis of the tissue employed.

### PURKINJE FIBERS

Carmeliet (159) has studied the movement of  $K^{42}$  across the membrane of sheep Purkinje fibers in media containing different concentrations of this ion; the half-time for  $K^{42}$  loss is sharply increased in low-K solutions and decreased in solutions containing an elevated K concentration. These observations are of particular interest since the membrane potential was approximately the same in both low- and high-K experiments. The demonstration of decreased K permeability in low-K solution is supported by the finding that membrane resistance is increased fourfold.

Studies of chloride permeability have been made on the same preparation (160). This ion makes only a small contribution to the resting membrane conductance, but its contribution is larger if the membrane is depolarized. Substitution of large anions for chloride causes little change in resting potential but does cause a prolongation of the action potential, an increase in rate of diastolic depolarization, and an increase in membrane resistance. Carmeliet concludes that distribution of chloride in Purkinje fibers is essentially passive. Hutter & Noble (161) performed similar studies on Purkinje fibers using anions which are presumably more ( $NO_3$ , iodide, nitrate, bromide) and less (methylsulfate or pyroglutamate) penetrant than chloride.

He also concluded that chloride makes only a small contribution to the resting membrane conductance.

#### ATRIUM

Goodford (162) found that the potassium concentration of contracting rabbit atria falls from an initial value of 167 mM to 140 mM after 1 hour and to 73 mM after 48 hours. These experiments were carried out at 35°C. and an extracellular K concentration of 5.6 mM. Holland (163) reported on the distribution of Na and K in different parts of rabbit atrium. Sodium is higher and K lower in the pacemaker region, and K concentration in this area does not increase in high-K solutions. Rayner & Weatherall (164) presented a large number of determinations of K concentration in right and left atria of rabbit hearts. In the same study they present data on inward and outward movements of  $K^{42}$  in quiescent and beating right and left atria and on the effects of acetylcholine on this movement. Both influx and efflux are more rapid in right atria; the rate of influx is strongly influenced by the mass of the tissue; stimulation increases both influx and efflux. In beating right atria,  $K^{42}$  exchange requires at least two exponential terms for its description; also, only part of the tissue potassium appears to be involved in the exchanges associated with activity.

Humphrey & Johnson (165) also have studied the exchange kinetics of  $K^{42}$  in rabbit atria; they conclude that the fiber K is in at least two compartments whose sizes depend on extracellular K concentration [see also (183)]. This study also presents data on effects of rate and of K concentration on  $K^{42}$  exchange. Persoff (166) has demonstrated that, when fiber K concentration in contracting rabbit atria is constant, determinations of early rate constants of  $K^{42}$  movement will differ depending on whether tissue or effluent is employed for counting. Levine and associates (167) present data on ion content of rabbit atria and changes caused by pyridoxal and other compounds. Contraction is associated with potassium loss and sodium gain.

Holland and his associates have published a number of papers on the movement of various ions across the membrane of fibers in rabbit atria. Unidirectional  $K^{42}$  fluxes have been related to the transmembrane action potential (168), and data are interpreted as indicating that the duration of the terminal phase of repolarization depends on active transport of Na, of K, or of both. Studies of  $Cl^{36}$  and  $Ca^{45}$  exchange during atrial fibrillation induced by a high rate of stimulation in the presence of acetylcholine have been reported (169), as well as the effects of ouabain on exchange of these two ions (170). Changes in influx and efflux of  $K^{42}$  during fibrillation have been related to the production of this arrhythmia (80). Quinidine ( $10^{-4}$  to  $10^{-5}M$ ) has been shown to cause a decrease in Na entry and a secondary decrease in K loss (171) as well as inhibition of active transport of K and Na; the decreased Na entry has been correlated with antiarrhythmic action. Also,  $K^{42}$  and  $Cl^{36}$  fluxes were compared during fibrillation induced by either acetylcholine or calcium (172).



## VENTRICLE

Brown *et al.* (173) showed that the potassium balance in the dog heart-lung preparation is not influenced by rate unless failure, caused by pentobarbital, is present. Interestingly, ouabain reverses failure and accelerates K loss. Conn & Wood (174) studied the effect of toxic concentrations of quinidine on K exchange in the isolated perfused dog heart; in these experiments arterial pressure was 40 to 60 mm. Hg, and fiber Na and K concentrations were unusual. In the same preparation (175), they studied Na exchange and distribution. Respiratory alkalemia causes an increase in K content and concentration in dog left ventricle while acidemia causes an increased content of K (176). Sodium is unchanged by either condition. In this study fiber concentrations of Na, K, and Cl were 12.5, 143, and 4 m.eq. per liter, respectively. Brown & Mowlem (177) showed a loss of K from dog ventricle following hypercapnia. Coronary sinus catheterization of human hearts shows that a loss of ventricular K is caused by epinephrine and norepinephrine (178). Mack and co-workers studied extraction of Rb<sup>86</sup> by rabbit hearts (179), and Cairns *et al.* (180) described the effects of acetylstrophanthidin on K<sup>42</sup> and Rb<sup>86</sup> kinetics in dog myocardium. Freed & St. George (181) reported on changes in myocardial Na and K during K deprivation and treatment with steroids. In relation to all of these reports, the findings of Cairns *et al.* (180) which relate to shunting of coronary flow should be considered.

## OTHER STUDIES

LeVein *et al.* (182) studied the effect of digitalis on potassium toxicity in the turtle heart, and Schreiber and associates (183) determined the effect of ouabain on potassium exchange in heart muscle mitochondria. Thomas (184) studied the effect of ouabain and ethylenediaminetetra-acetic acid on Ca<sup>45</sup> movements and contracture of the frog heart; he finds no change in Ca<sup>45</sup> uptake unless ouabain causes contracture.

The effects of injection of K, Li, Ca, and Mg into the coronary arteries of dogs have been reported (185), as well as electrocardiographic changes caused by hypertonic NaCl (186) and changes in the electrocardiogram and plasma electrolytes caused by Rb (187). Tibbs & Berman (188) studied the effect of extracellular sodium concentration on the action of acetylcholine. Several studies of electrocardiographic alterations related to potassium concentration have appeared (189, 190). In many of the studies of ionic fluxes, conclusions concerning the ionic basis of changes in transmembrane potential seem unsupported by adequate experimental observations.

## METABOLISM

## OXYGEN UPTAKE

The oxygen uptake of cardiac muscle both in isolated preparations and in the heart *in situ* has been studied by a variety of methods. Lee used the oxygen polarograph to measure oxygen uptake of isolated cat papillary



muscle (191; see also 192) at rest, as a function of resting length, and as a function of frequency of contraction. Whalen (193) has carried out similar studies on cat papillary muscles and trabeculae carneae from rat ventricle using manometric determination of oxygen consumption. Both find that the  $Q_{O_2}$  increases as a function of resting length and conclude that this effect is independent of changes in muscle diameter and thus is not dependent on limited diffusion. Both find that  $Q_{O_2}$  increases with increased frequency of contraction whether contractile tension is increased or decreased by the higher rate. Lee (191) finds a  $Q_{O_2}$  for cat papillary muscle of 1.16; Whalen (193) finds a value of 2.7 for cat papillary muscle and 6.0 for rat trabeculae carneae. The increase in  $Q_{O_2}$  amounts to 0.8%/1% in length (193). Lee *et al.* used the same method to study the effect of ouabain on cat papillary muscle (192) and find that tension is increased, followed by an increase in oxygen consumption. The change in oxygen uptake was the same for quiescent and contracting muscles. The concentration of ouabain employed clearly was toxic. Pyruvate increased both  $Q_{O_2}$  and tension while succinate increased  $Q_{O_2}$  but diminished tension (324 to 326). In contrast to the findings of Lee and Whalen on isolated preparations, Monroe & French (194) studied the oxygen consumption of the dog heart during fibrillation and after citrate arrest; distention of the fibrillating ventricles caused a great increase in oxygen uptake while distention of the ventricles during arrest caused no change in this measurement. Sayen and co-workers (195) investigated the effect of norepinephrine on myocardial oxygen consumption using the polarograph and obtained data which suggest that this agent causes an increase in the relative blood supply and a shift to anaerobic metabolism. Whaley *et al.* (196) has shown that both *l*-thyroxine and *l*-triiodothyronine, when injected into thyroidectomized rats, increase the oxygen consumption of cardiac muscle. The myocardium of infant rats was found more resistant to anoxia than that of mature animals (197). Oxygen consumption in the fibrillating dog heart has been studied and related to autonomic control of coronary vascular tone (198). The oxygen consumption of slices of rat ventricle, from manometric determinations, was found to be influenced by prior cooling of the animal; control  $Q_{O_2}$  was 8.5 and, after 3 hours at 21°C., 5.7 (199). This change was associated with a decrease in myocardial glycogen and was prevented by addition of glucose as a substrate. Oxygen consumption of dog hearts was found to be increased in the presence of hypertrophy caused by aortic insufficiency (200).

#### METABOLIC TURNOVER AND MITOCHONDRIA

Fleckenstein *et al.* (201) have reviewed studies from his laboratory of the high-energy phosphate compounds in heart muscle combining radiophosphorous and paper chromatographic techniques. In addition to values for the various phosphate fractions, data on isotopic incorporation into the three sites in ATP and into creatine phosphate were obtained under several conditions. An interesting finding is a slightly greater level of energy-rich

phosphates in quiescent as compared to beating rabbit atria, while radiophosphate incorporation (as in skeletal muscle) is not at all different under the two conditions. Similar results have been reported by Tsuboi, Buckley & Zeig (202). Both groups emphasize the possibility that an apparent maximum for orthophosphate incorporation may be caused by a limiting cell or mitochondrial permeability for this ion. Fleckenstein's group appears to have solved this problem (203). Measurements of incorporation of the immensely more diffusible  $\text{H}_2\text{O}^{18}$  into phosphate fractions in frog rectus have shown a good-sized increase with tetanic activity. The method has not, as yet, been applied to heart muscle. Creatine phosphate content declines linearly with increasing stroke volume in the guinea-pig heart-lung preparation, according to Hochrein & Doering (204), while the orthophosphate fraction reciprocally increases; other high-energy phosphates are not affected, the ATP fraction being reduced only during failure; the fall in creatine phosphate with increasing work is steeper under conditions of arterial pressure loading than under those of volume loading.

Ramirez (205) followed spectrophotometric evidence of changes in the oxidation-reduction levels of respiratory chain enzymes which occurred with contraction of frog and toad ventricular muscle. Pyridine nucleotides, flavoprotein, and cytochromes *b* and *a<sub>3</sub>* were oxidized during activity, while cytochromes *c* and *a* were reduced. The rapid onset and disappearance of these changes with the start and cessation of contraction are seen as a further indication that the respiratory chain, rather than the ATP-creatine transphosphorylase system (as in skeletal muscle), represents the predominant mechanism for ATP resynthesis in the heart. Myocardial creatine phosphate measurements have been found to be increased after glucose infusion (206). Such measurements are probably falsely low because of contracture that takes place in the freezing medium. Producing arrest with potassium citrate and with acetylcholine before plunging the tissue into the freezing agent, Lee *et al.* (207) have obtained values ( $10\mu\text{M}$  per gm.) comparable to those of Wollenberger *et al.* (208), who employed an ingenious method (209) for totally freezing the tissue in much less than one second.

The coronary dilatation produced by adenosine is not attributable to its metabolic product, inosine (210); dinitrophenol-induced dilatation may be related to release of adenosine from myocardial cells. Schreiber (183), studying  $\text{K}^{42}$  exchange in heart mitochondria, concluded that mitochondrial potassium cannot be a large part of the half of myocardial potassium that he previously found to be slowly exchangeable. Ulrich (211) demonstrated mitochondrial accumulation of sodium and potassium; the latter was increased by the addition of ATP and oxidizing substrate to the medium. Agents uncoupling oxidative phosphorylation inhibited potassium transport, but only at relatively high levels; ATP, but not creatine phosphate, produced reaccumulation of mitochondrial potassium in the presence of uncouplers (212). Ethylenediaminetetra-acetic acid has a stimulatory effect upon mitochondrial respiration, not related apparently to its calcium-mag-

nesium or iron-chelating effects (213). Corticosterone was found by Jensen (214) to inhibit pyridine nucleotide oxidase, and acetylcholine was shown by Medina & Bacila (215) to reverse thyroxine uncoupling of oxidative phosphorylation in heart sarcosomes. Burstone (216) has histochemically demonstrated sarcosomal localization of cytochrome oxidase in the human heart. Helander & Emmart (217), using a fluorescing antimyosin which they had prepared, demonstrated myosin in myofibrils of beef Purkinje fibers.

#### HEART FAILURE AND DIGITALIS

The view is now widespread that heart failure, with the exception of some situations involving frank metabolic derangement, represents a disturbance in utilization, rather than in production, of metabolic energy (218, 219). Some disagreement with this notion has recently been expressed. Szekeres & Schein (220) demonstrated glycogen depletion, decreased high-energy phosphate fractions, and decreased phosphate ester synthesis (homogenates) for rat ventricle in "acute failure" (after a half hour of aortic constriction). Other studies making a similar point (221, 222) will shortly be reported in detail. Gertler (223) has described a method of producing heart failure experimentally (aortic constriction to one-third initial diameter) in eight to twelve days; histologic sections show changes characteristic of congestive heart failure, rather than the usual fibrosis. Canine cardiac actinomycin, as characterized by Davis *et al.* (224) from sedimentation velocity, viscosity, and adenylyl triphosphatase activity, was not found to be altered in congestive heart failure (pulmonic stenosis), except for an abnormal sedimentation component, probably related to the preparatory technique. An increased RNA and decreased DNA (225) were found in hypertrophied dog ventricle (aortic cusp avulsion). According to Kien & Sherrod (226), digoxin, administered to normal dogs, increases glucose utilization and turnover in the glycolytic and tricarboxylic cycles, augmenting the contribution of glucose to the carbon dioxide production, an effect attributed to a digoxin-induced increase in permeability of the heart cell to glucose which is similar to that postulated for insulin (227). Cardiac glycosides in toxic doses have a "mild uncoupling action" on oxidative phosphorylation by heart mitochondria (228). Holland & Klein (229) published a monograph on heart failure and the action of cardiac glycosides; this work includes a summary of many of their own studies and viewpoints.

#### HYPOXIA AND ISCHEMIA

In some instances, the effects of ischemia have continued to be confused with effects of hypoxia. Direct records from the specialized conducting system *in situ* have been used to study the different effects of hypoxia and ischemia on the dog heart *in situ* (230). Ischemic effects on atrioventricular conduction and ventricular muscle were related to changes in lactic acid production, potassium and ATP content, and pH of the heart (126). Hearts from thyroidectomized rats were found less sensitive than normals to the

effects of combined hypoxia and hypothermia (231). The mechanism of cardiac damage by anoxia has been related to production of lactic acid and intracellular proteolysis; myocardium from infant rats differs from adult muscle (232). Arrest caused by lack of oxygen or substrate is irreversible and results from cellular damage, while arrest resulting from lack of perfusion is caused by extracellular potassium accumulation and is reversible (233). These results show great similarity to well-known findings obtained on nerve. ECG changes in dogs have been found to be reversible after 40 minutes and irreversible after 60 minutes of ischemia (234). Abolition of ischemic changes in the ST segment by reduced  $po_2$  in inspired gas has been attributed to the existence of a myocardial oxygen gradient (235). Effects of regional ischemia and norepinephrine on the RS-T segment and baseline of electrograms recorded with direct-coupled amplifiers were recorded (236). An electron-microscopic study of changes in the myocardium resulting from ischemia recently appeared (237). Studies of ECG changes induced by exercise have continued (238, 239). Ischemia and hypoxia are associated with an increase in myocardial orthophosphate at the expense of high-energy phosphate (201, 240, 241), as well as a decrease in high-energy phosphate synthesis (201, 242) and glycogen content (241, 243).

#### OTHER EFFECTS

Glucose, otherwise essential for the survival of embryonic heart tissue culture, has been found to be replaceable by seven other monosaccharides (244). Coronary perfusion of dog hearts with gaseous oxygen was carried out with persistence of contractile activity for periods of up to eight hours (245). From measurements of the absorption of long-wavelength radiation by supercooled tissue samples, Hopkins (246) obtained results from frog ventricle, which he feels may indicate the binding of intracellular water with contraction.

#### MECHANICAL PROPERTIES OF HEART MUSCLE

##### STAIRCASE, LENGTH-TENSION, ETC.

Braveny & Kruta (247), in terms of extrasystolic, postextrasystolic, and staircase responses of the guinea-pig atrium, have more quantitatively defined the old unifying idea that two opposing processes are generated with each contraction which modify the strength of the following beat. The term "restitutive" process is applied to the fact of increasing contraction at increasing intervals after the previous beat; an opposite "potentative" effect declines with increasing interval. As evidence for the identity of the potentiating processes in staircase and in postextrasystolic beats, the authors offer the fact that maximum "potentiation" occurs at the interval corresponding to the rate for staircase peak at each of three different temperatures. The unusually long time-course they assign to the "restitutive" process is consistent with their view that poststimulation and rest potentiations might also fit into the simple restitution-potentiation scheme. Some uncertainty

may exist as to whether their results, obtained from isotonic recordings, would be quantitatively reproduced under isometric conditions, in view of recent indications (248, 249) that isotonic and isometric contractions of skeletal muscle are more dissimilar than tension and length differences would suggest they should be.

Speirs (250) has studied the potentiating effect upon rat ventricle preparations (isometric recording) of rapid stimulation followed by a rest pause. Two findings, suggesting a maximum attainable contraction, may be related to present views on the role of calcium (see the section on excitation-contraction coupling): the stimulation-rest potentiation is not added to by a post-extrasystolic beat; the increased basal rate contractions in the presence of high calcium or low sodium are not further potentiated by the rapid stimulation-rest sequence. Wallon *et al.* (251), using a method of tension recording from a limited region of isolated rabbit heart, found that the increased contraction in low-sodium solutions is not further augmented after a period of rapid stimulation. A stimulation-rest procedure employed (252) with rat papillary muscle, but with stimulation rates (10 to 40 per sec.) not followed by the muscle, evoked a potentiation which the author suggests may be caused by catechol amine release since spontaneous activity of the preparation frequently followed such rapid stimulation. The anomalous behavior of rat heart (decreasing contraction with increasing frequency) is emphasized by Kruta & Stejskalova (253), who interpret this as an unusually weak potentiation process relative to restitution, and by Hoffman & Kelly (254), using the papillary muscle. The latter authors show data which appear to rule out a variety of commonly considered factors: oxygen diffusion limitation, intermediate substrate depletion, catechol amines, weak postextrasystolic or calcium potentiation, prolonged restitution phase, rest potentiation, potassium loss. A slow decline in contractile tension with time appears to involve multiple factors (255).

The restitution process and postextrasystolic potentiation in the dog heart *in situ* (with right ventricular pressure recording) were shown by Siebens *et al.* (256) to be very similar to their counterparts in isolated preparations and quite independent of effects arising from recovery of excitability and ventricular filling. The same conclusions are reached by Meyler (257). A second type of "staircase" phenomenon, not related to rate or interval of contraction, was described by Rosenblueth *et al.* (258) in the isolated dog heart, mounted in a cardiometer, perfused and filled by blood from a donor animal. An increased right ventricular stroke volume in response to an elevated filling pressure persists for at least 30 seconds after the filling pressure has been reduced to its previous level; they relate this observation to the initial increase in the work of the heart. A similar effect, however, has been demonstrated by Walker (259) with isometrically mounted dog papillary muscle: in the course of stepwise increases and decreases in quiescent length, contractile tension at any given length was greater during the releasing step than during the stretching step. Quiescent tension was greater, for

any given length, during the stretching step. Rosenblueth *et al.* (260) studied contraction of the dog ventricle *in situ* under isotonic, isometric, and after-loaded conditions. Using a sensitive recorder of localized precordial movements, Suh & Eddleman (261) demonstrated the frequent occurrence of systolic bulging of ischemic heart muscle during the early course of myocardial infarction in man. Harrison & Hughes (262) recorded transient bulges occurring during anginal attacks.

Summation and tetanus were obtained (263) from human papillary muscles *post mortem*, in association with an electrical refractory period considerably shorter than the contraction time. It is tempting to relate this fact to the observation of Griggs *et al.* (264) that hypoxia and fluoride ion cause ventricular muscle to be shorter and less distensible. Buckley & Zeig (265), in a study of acute failure of the dog's left ventricle, note the decreased distensibility (increased "impedance") of the ventricular muscle, as well as the slower contraction and relaxation. Salisbury *et al.* suggest that decreased myocardial distensibility may arise, in part, from tissue edema (266) or from engorgement of the coronary circulation (267). Hennacy & Ogden (268) have measured the time-course of nondistensibility ("renitence") of frog ventricle during diastole; renitence is increased by a combination of hypoxia and acidity, among other things.

Two unusual and disturbing papers have appeared concerning grossly deleterious effects of experimental conditions and apparatus on contraction. McCarty & Shideman (269) reported that cat papillary muscles show spotty to extensive histological changes (pyknotic nuclei, absent striations) after being subjected to three or four grams of tension for six and one-half hours. Isotonic contractions steadily declined; isotonic contraction resulted in the more severe morphologic changes for loads of three grams or greater. Isometric behavior was the same for all maintained tensions. Meyler *et al.* (270) presented evidence that an elutable component of some types of polyvinyl chloride tubing, probably those containing an organic tin stabilizer, produces a cumulative and irreversible decline in contractile amplitude. Paff *et al.* (271) have developed a method for recording contractile activity from the embryonic chick heart. Selye (272) has published a book on his work with experimental cardiac necrosis.

#### IONS AND EXCITATION-CONTRACTION COUPLING

Sten-Knudsen (273) has published some careful experiments supporting the relation between membrane depolarization in skeletal muscle and the initiation of contraction, including a refutation of a recent assertion (274) that longitudinal current flow inside the fiber also plays a role. Recording from very short segments of right ventricular trabeculae carneae (sheep and calf), Kavalier (275) demonstrated a prolongation of near-peak isometric tension in response to extended (by current flow) membrane depolarization (2 sec.). This correlation does not hold for dog atrial muscle (276; see also 255). Reichel & Bleichert (277) summarized their findings that the active



state (quick release) in frog ventricle and turtle atrium has a plateau and time-course very similar in shape to that of the corresponding action potential, though noticeably longer in duration. Vaughan Williams (8) has studied the negative inotropic effect of acetylcholine and vagal stimulation on rabbit atrial preparations and concludes that the cholinergically induced shortening of the action potential duration is not per se responsible for decreased force since an increased contraction is obtained immediately by stimulation during the period of arrest, while the action potential is maximally shortened. The possibility that rest potentiation may be additionally involved here would not appear to bear on the conclusion, since the times to peak of the weakened and of the restored contractions do not appear to be shortened [cf. (276)]. In another paper on the relation between rate and contraction (9), this author shows that it is the more frequent excitations rather than the evoked contractions which are responsible for the augmentation of contractility. He also lays some emphasis on the increasingly pronounced negative after-potentials which appear to be appended to a basic early action potential of constant form, as indicating the process that is responsible for rate augmentation of contraction.

With an experimental design which rapidly increased the extracellular calcium concentration by coronary injection in the cold turtle heart during a 10-sec. action potential, Weidmann (278) has demonstrated that calcium so injected during the action potential plateau increased the contraction strength of that beat. Niedergerke (279; cf. 280) published an important addition to his studies on the role of calcium in excitation-contraction coupling. Potassium chloride contracture and the augmented contraction in the presence of a decreased external sodium concentration are each associated with an increase in the radiocalcium uptake by frog ventricle strips. Sulser (281) has reported that, while strophanthin increases the net potassium efflux from guinea-pig ventricle (with Langendorf perfusion), the inotropic effect of increased calcium is unaccompanied by any change in potassium transport. He suggests that the mechanism common to digitalis and calcium inotropy may be an increased calcium transport into the fibers, a supposition supported by Holland & Sekul (170) who have observed an increased calcium uptake, caused by administration of ouabain, by the rabbit atrium [see also (184)]. Calcium uptake is further increased in the presence of higher calcium concentrations and decreased at a potassium concentration of 8.1 mM, one which prevents ouabain contracture. The contracture of frog skeletal muscle in potassium-free Ringer, on the other hand, is associated with an increased calcium uptake (282).

#### HORMONES, DRUGS, METABOLITES, POISONS

Myocardial catecholamines have attracted much interest from several points of view. The results of Furchgott *et al.* (283) from guinea-pig atrium clarify considerably the role of catechol amine release in the potentiation of contraction (284, 285) caused by suprathreshold stimulation; dichlorois-



proterenol, which abolishes the positive inotropic effects of epinephrine and norepinephrine, also abolishes potentiation caused by suprathreshold stimulation but does not affect postextrasystolic or poststimulation potentiation elicited by threshold-range stimuli [cf. (252)]; atrial preparations from reserpinized guinea pigs respond with an increase in contraction to epinephrine and norepinephrine [see also (286)], but not to suprathreshold stimulation; dichloroisoproterenol blocks the positive inotropic effect of nicotine. Papillary muscles from reserpinized or sympathectomized cats show markedly reduced contractions (287). Reserpine and a variety of other rauwolfia alkaloids (288) have been shown to reduce the myocardial content of catecholamines (287, 289) or of norepinephrine alone (288). Monoamine oxidase inhibitors increase myocardial catecholamines (290, 291), and this is reflected in a positive inotropic effect (292). The positive inotropic effects of tetraethylammonium, nicotine, and acetylcholine on several atropinized heart preparations are markedly reduced after reserpine administration, when catecholamines markedly increase contraction (293). Direct evidence of catecholamine release from atropinized hearts caused by acetylcholine has also been obtained (294); a positive inotropic effect of acetylcholine on rat ventricle has been reported (295). Dichloroisoproterenol, at less than blocking concentrations, has a positive inotropic effect on cat papillary muscle (296). Catechol isomers and related compounds show positive inotropic effects (297). The increase in contraction by epinephrine remains unchanged after addition of hydrocortisone or aldosterone to heart-lung preparations from normal or adrenalectomized dogs (298); corticosteroids, however, restore contractility to the weakened heart-lung preparations from adrenalectomized rats (299). Regional differences in catecholamine contents have been found in the hearts of several species (300). Norepinephrine is decreased in the heart in the presence of renal insufficiency (301). Chick embryo hearts show the same response to epinephrine and to acetylcholine before and after nervous innervation occurs (302). Trautwein *et al.* (303) presented electrophysiological evidence that acetylcholine is released spontaneously in the beating-dog atrium.

The augmentation (or lack of augmentation) of contractile force by several sympathomimetic amines has been correlated with their abilities to increase myocardial phosphorylase-*a* activity in the isolated rat heart (304) and in the dog ventricle *in situ* (305); dichloroisoproterenol blocks the catecholamine effects on contractile force and on phosphorylase-*a* activity (305). The correlation between phosphorylase-*a* activity and contractile amplitude cannot be successfully generalized to include all conditions affecting the latter, as has been emphasized by Belford & Feinleib (306). Manukhin & Vuznikov (307) have described the inotropic effect of 1 per cent urea (in Ringer) on the sympathetically innervated frog ventricle preparation (Straub cannula). There is a reversible abolition of the usual contractile response to sympathetic stimulation, or catecholamine addition, in the presence of urea-Ringer. Byvshuk (308) reported that the augmentation of the contraction of

rat heart by norepinephrine and epinephrine is accompanied by quite different reductions in tissue glycogen [cf. skeletal muscle (309)]. Adrenergic effects on cardiac contraction have again been characterized as a shortening of the time-courses for both contraction and relaxation (310). Sayen *et al.* (195) obtained results indicating that epinephrine also brings about more synchronous contraction.

Rather consistent contraction-promoting effects of adrenal corticosteroids have been recently shown (299, 311). Much of the evidence indicates a protective effect against experimentally occurring deterioration; possible mechanisms are discussed by Tanz (311). King *et al.* (312) found a decrease in actinomyosin content to be associated with the reduced contractility of ventricular muscle from ovariectomized rats; both are partially restored by estrogen administration. Read & Nehorayan (313) conclude that the decreased cardiac contraction and creatine loss (rabbit) occurring in vitamin E deficiency are only uncertainly related to diminished myocardial creatine phosphokinase activity. Beznák (314), using pituitary powder and some newer, more adequately characterized fractions, has restudied the dependence of ventricular function and of the hypertrophy caused by aortic constriction upon adeno-hypophyseal hormones; hypodynamic circulatory function in hypophysectomized rats appears to be secondary to decreased metabolism and is partially corrected by administration of TSF fraction; the hypertrophic response requires the growth hormone fraction. Hajdu & Leonard (315) have published an extensive review on the mechanisms of action of cardiac glycosides. Reviews by Krantz & Ling (316) and by Somlyo (317) have also appeared. Studies of glycoside effects are cited in other parts of this review. Hillyard & Procita (318) have presented further details on the irreversible failure of the contraction of heart muscle induced by the alkaloid ryanodine; interestingly, a considerable decline in contractile strength occurs at a time when there is no detectable decrease in high-energy phosphate components.

Guanosine, inosine, thymidine, and uridine have been reported to improve the failing left ventricle of the dog, adenosine and cytidine to aggravate the failure (319). Cytidine has been reported to exert a positive inotropy in the isolated perfused toad heart (320), thiamine mono-, di-, and triphosphate a negative inotropy on frog heart (321, 322), and glucose-1-phosphate a positive inotropy on rabbit heart (323). Levine *et al.* (167) attempted to correlate contractile changes induced by several metabolites and poisons with tissue sodium and potassium changes. Covin & Berman (324) have shown the inotropic effect of arsenate on rat ventricle strips to vary with the substrate used: marked positive inotropy in glucose and in substrate-free media; slight positive to negative inotropy in pyruvate. These differences are said to be correlated with the effect of arsenate on the rates of oxidation of the substrates used (325). Similar substrate-dependent differences in inotropy were found for fluoride (326) and ouabain (192). Venturi &

Schoepke (327) have studied the negative inotropic effects of a variety of Krebs-Meyerhof substrates on rat atrium; they incline to the view that these substances have their effect through calcium binding because of evidence that the effects of high calcium in the presence of these substrates do not correspond to the concentrations used. Separate humoral factors from spleen and liver are thought to play a role in temporarily sustaining myocardial contractility in a modified heart-lung experiment; hypoxia does not influence the release of these factors into the lienohepatic venous system (328). The cardioactive effect of extracts of freeze-dried spleen is not explicable in terms of their choline, acetylcholine, catecholamine, histamine, or 5-hydroxytryptamine contents (329).

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## EXCITATION AND CONDUCTION<sup>1,2</sup>

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The following review discusses current aspects of certain topics, falling within the scope of the assigned subject, which are of particular interest to the reviewers. The authors wish to apologize for the omission of many reports equally as important as those quoted.

### MEMBRANES

The concentration of several species of ions is different inside and outside cells. For example, nerve and muscle cells contain higher concentration of  $K^+$  and lower concentration of  $Na^+$  than external fluids. Generation and maintenance of this difference of concentration require energy which must be supplied by metabolic processes.

According to presently accepted views, resting and action potentials arise as a consequence of the different concentration of ions inside and outside excitable cells. Since ions leak through the membrane following their concentration gradient, both at rest and (more rapidly) during activity, long-term maintenance of the resting potential and of the ability to produce action potentials requires metabolically driven transport of ions in the opposite direction.

*Active transport of ions across membranes.*—Several papers which appeared during the period covered by this review deal with movement of ions against their concentration gradient—"active transport".<sup>3</sup>

It was found that metabolic inhibitors such as dinitrophenol (DNP) and cyanide (CN) rapidly decrease the concentration of adenosine triphosphate (ATP) and of arginine phosphate in squid axons (30, 32) and also reduce the active transport of  $Na^+$  and  $K^+$  across the membrane (107). Injection of either ATP, arginine phosphate, or adenosine diphosphate (ADP) in poisoned axons partially restores  $Na^+$  extrusion. The extrusion of  $Na^+$  from normal axons does not increase if the concentration of high-energy phosphates is increased (31, 123). The inhibitors which affect  $Na^+$  extrusion also reduce  $K^+$

<sup>1</sup> The survey of literature pertaining to this review was concluded June 30, 1960.

<sup>2</sup> Among the abbreviations used in this chapter are: EPSP (excitatory postsynaptic potential); GABA (gamma-aminobutyric acid); IPSP (inhibitory postsynaptic potential).

<sup>3</sup> The term "active" is used in relation to ion movements when this movement requires work against the electrochemical gradient. Movements along the concentration gradient are often referred to as "passive" in this context. However, when discussing nerve impulses and related phenomena the term "activity" is often used to designate events accompanied by changes in the electric constants of the membrane, although these result in movement of ions along their concentration gradient.

absorption (107). However, when  $\text{Na}^+$  efflux is reduced to one-twentieth of its initial value,  $\text{K}^+$  influx is decreased only to one-seventh (107). The movements of  $\text{Na}^+$  and  $\text{K}^+$  against their concentration gradient are apparently not so tightly coupled as has been proposed [108 (p. 28); see also 111, 123, 164)].

It is possible that the coupled mechanism is predominant in normal axons but not in poisoned ones. When  $\text{Na}^+$  efflux exceeds  $\text{K}^+$  influx, the question arises as to which ion balances the electrical charges; so far there is no answer to this question. Keynes (123) suggests that two mechanisms operate in active transport: in one the transport of  $\text{Na}^+$  and  $\text{K}^+$  are coupled, in the other they are not. Energy for these movements is probably supplied not only by ATP but by more than one compound, including arginine phosphate.

Considering only the coupled transport system, Caldwell (32) has worked out a scheme based on models which had been proposed previously by other authors (108, p. 29). The potassium ion is supposed to enter the cell together with a carrier molecule; inside the cell the carrier undergoes a chemical change which converts its previous affinity for  $\text{K}^+$  to a specific affinity for  $\text{Na}^+$ . As this second compound reaches the outside of the cell, the carrier is converted again to the  $\text{K}^+$ -carrying form and thus becomes available for  $\text{K}^+$  transport. Adenosine triphosphate and arginine phosphate appear to be required sources of energy for this cyclic process. Because  $\text{Mg}^{++}$  has been found to affect the action of ATP, Caldwell suggests that a complex may be formed between ATP and  $\text{Mg}^{++}$  in the cycle of energy exchange.

*Origin of membrane potentials.*—Bernstein (19) suggested in 1902 that the resting membrane is selectively permeable to  $\text{K}^+$  and that membrane potential is controlled solely by difference of concentration of  $\text{K}^+$  on the two sides of the membrane.

Boyle & Conway (20) suggested that the membrane of resting muscle fibers is permeable to both  $\text{K}^+$  and  $\text{Cl}^-$  and that the two ions are distributed according to a Donnan equilibrium. Working with isolated muscle fibers of frogs, Hodgkin & Horowicz (110) obtained results in agreement with Boyle & Conway's hypothesis. According to their measurements, the electrical properties of resting muscle membrane in Ringer's fluid can be represented by a circuit diagram with two electromotive forces ( $V_K$  and  $V_{Cl}$ ) and two resistances ( $R_K$  and  $R_{Cl}$ ) in parallel. The value of  $R_K$  is about ten kilo-ohms and that of  $R_{Cl}$  is about five kilo-ohms for one square centimeter of membrane. These values are in agreement with later estimates which ascribe to  $\text{Cl}^-$  conductance 68 per cent of the total conductance of a resting muscle fiber (112). Confirming previous findings by Katz (121), it was found however that the permeability of the membrane to  $\text{K}^+$  is high (about  $8 \times 10^{-6}$  cm. per sec.) for movement towards the inside of the membrane but is much lower (about  $0.05 \times 10^{-6}$  cm. per sec.) for movements in the opposite direction (anomalous rectification). Permeability of  $\text{Cl}^-$ , on the other hand, appears to have a constant value of about  $4 \times 10^{-6}$  cm. per sec. which is independent of direction of current. The mechanisms responsible for this rectifying property

of muscle membrane are not understood at the present time and, in fact, the phenomenon "is particularly puzzling because the rectification is in the opposite direction to that required to explain the recovery of potential and the loss of potassium during the impulse. Presumably the behavior of the membrane at short times is different from that in the steady state, but on this point there is little experimental evidence" (110, p. 156). Adrian (6) has shown that if a muscle is loaded with potassium chloride and then is transferred into a  $\text{Cl}^-$ -free solution, membrane potential is initially  $+60$  mv. (inside positive) and drifts progressively to  $+40$  mv. At this point, some fibers drop suddenly to a potential of  $-20$  mv. while others oscillate spontaneously between  $+40$  mv. and  $-20$  mv. Resistance is about 10 times lower when the potential is  $-20$  mv. than when it is  $+40$  mv. This phenomenon is probably closely related to the results obtained by Tasaki (178, 179), Stämpfli (172), and others and mentioned later in this article (p. 360). The proposed interpretation is that in the conditions of the experiment,  $\text{K}^+$  permeability varies suddenly between two values, while  $\text{Cl}^-$  permeability probably remains constant.

Work with radioactive tracers (109) showed that  $\text{Na}^+$  moves across the resting muscle membrane more rapidly than  $\text{K}^+$ . For each ion, the rates of influx and efflux are approximately equal. It should be noted that interpretation of these results is complicated because the measurements include self-exchange, active transport, and perhaps movement in combination with anions.

Rubidium and caesium in the outside fluid reduce the membrane potential of the muscle membrane (168), acting as though the membrane were somewhat permeable to these ions. A recent study performed by Stämpfli (172) showed that the membrane potential of Ranvier nodes in frog nerves is affected by changes of  $\text{K}^+$  concentration in the manner predicted by Bernstein's hypothesis (19) [see Huxley & Stämpfli (113)] only if the dissected fibers are not in good condition. With carefully dissected, fresh fibers, major changes of external potassium concentration evoke only small changes of potential, but the potential may suddenly become sensitive to the external concentration of  $\text{K}^+$  following a number of experimental procedures. Maintenance of the  $\text{K}^+$ -insensitive condition of the membrane is favored by  $\text{Ca}^{++}$ , by  $\text{Cl}^-$ , and by hyperpolarization; membrane resistance is then considerably higher than it is in the  $\text{K}^+$ -sensitive state. These results indicate that the resting potential of Ranvier nodes is not always controlled by  $\text{K}^+$  concentration. According to Stämpfli the results "can be explained in terms of Hodgkin's conception of the interaction of a voltage-dependent potassium conductance  $g_K$  and a constant chloride conductance  $g_{\text{Cl}}$  at resting potential level" (172, p. 283). However, this interpretation is not supported by the finding that increase of external concentration of  $\text{Cl}^-$  decreases membrane potential. A further complication is created by the observation that an excess of external  $\text{Cl}^-$  lowers membrane resistance if membrane potential is high, but increases resistance if membrane potential is low.

*Two stable states.*—Two membrane conditions characterized by different electromotive force and resistance have been analyzed by Tasaki (179) and Hagiwara & Tasaki (178). In both conditions, "the membrane returns directly to its original state, after a weak perturbing pulse causing a small passive change in the membrane is withdrawn" (179, p. 313); these conditions are thus referred to as "stable states". These two states of the membrane are probably the same conditions observed by Stämpfli (172) [see also Stämpfli (171), Mueller (148, 149), and Segal (163)]. According to Tasaki's results, in the "lower steady state", membrane potential is 20 to 30 mv. larger and membrane resistance is 1.2 to 3 times greater than in the "upper steady state". When the nerve fiber is immersed in a solution containing high concentration of  $K^+$ , transition between the two states can be brought about by hyperpolarizing currents. With high external  $K^+$  and with a critical intensity of steady hyperpolarizing current, spontaneous transitions between the two states could be demonstrated (180). Comparable phenomena are also found to occur in the iron wire model. Gradual, rather than abrupt, transition between the two states may be obtained if some areas of the membrane are in one state and other parts in the other state (p. 366). The view explaining gradual changes as the result of abrupt transitions in small areas is reconsidered by FitzHugh (60; see also 117, 139) in a theoretical study on the prolonged spikes which can be obtained in nerves under tetraethylammonium (TEA) (169, 178, 186). FitzHugh found that the Hodgkin & Huxley equations, modified by multiplying the time constant of  $K^+$  activation by 100 and that of  $Na^+$  activation by one-third duplicate the experimental action potentials obtained under tetraethylammonium but reproduce the conductance measurements rather poorly. Thus, FitzHugh points out (60, p. 92):

Tasaki and Hagiwara's (1957) objections to Hodgkin and Huxley equations on the basis of their conductance measurements . . . still have some validity for the modified Hodgkin and Huxley equations. . . . However, in view of the good agreement of the plateau action potential curves and of abolition and refractoriness, it may still be worthwhile to look for better modifications of the equations capable of reproducing the experimental membrane conductance measurements.

Tasaki's description of the results in terms of two stable states is acceptable but the assumption that intermediate conditions are caused by the presence of patches of membrane in one state and of other patches in the other state "is not necessary in order to give plateau-type action potentials, since spatial uniformity for all variables has been assumed" (60, p. 893) in FitzHugh's calculations.

*Calcium and anesthetics.*—It has been known for a long time that  $Ca^{++}$  deprivation decreases the threshold or evokes spontaneous firing of peripheral nerves. Frankenhaeuser & Hodgkin's 1957 work on squid axons (70) established that  $Na^+$  conductance becomes more sensitive to depolarization under  $Ca^{++}$  deficiency. The results obtained on the node are consistent with the same view [Frankenhaeuser (69)]. Magnesium has qualitatively the same effects as calcium (71). Huxley (114) has used Hodgkin & Huxley's (1952)



formulation to calculate the effects of  $\text{Ca}^{++}$  deprivation, based on the finding that the effects of reducing  $\text{Ca}^{++}$  on  $\text{Na}^+$  and  $\text{K}^+$  permeabilities are the same as those brought about by depolarization. The damped or undamped oscillations which are found to occur are in good agreement with experimental results obtained in real axons.

Work on squid axons (167) dealing with a comparison of the effects of  $\text{Ca}^{++}$  and anesthetics (procaine and cocaine) were interpreted as confirming the results mentioned above (70). It was found that both anesthetics and  $\text{Ca}^{++}$  increase threshold by decreasing the dependence of  $\text{Na}^+$  conductance on depolarization. However, while anesthetics decrease  $\text{Na}^+$  and  $\text{K}^+$  conductance at all levels of membrane potential,  $\text{Ca}^{++}$  decreases  $\text{Na}^+$  conductance for small depolarization but increases it for depolarizations greater than 40 mv. The authors suggest that  $\text{Ca}^{++}$  reduces the number of sites available for passage of monovalent ions at low depolarization but increases the number of sites at higher depolarization. To justify this Shanes *et al.* suggest (167, p. 800) that

(a)  $\text{Ca}^{++}$  occupies sites that normally become available to the monovalent ions with activity; (b) in its interaction with the membrane,  $\text{Ca}^{++}$  causes more such sites to appear and occupies them prior to activity; and (c) when depolarization occurs, particularly above a certain level,  $\text{Ca}^{++}$  is removed from these sites, which then function for the transfer of monovalent ions. The local anesthetics, on the other hand, can cause only a reduction in the availability of sites during depolarization.

Results of other experiments suggest that local anesthetics reduce permeability to  $\text{K}^+$  and  $\text{Na}^+$  by affecting only the outermost layer of nodal membrane (165).

The outflux of  $\text{Ca}^{++}$  is much quicker from tendon than from muscle (166). With either tissue, outflux is quicker if the outside solution contains  $\text{Ca}^{++}$ . The slow component of the outflux from muscle is ascribed to that fraction of  $\text{Ca}^{++}$  (about  $\frac{1}{3}$ ) which had penetrated the muscle fibers. It is concluded that  $\text{Ca}^{++}$  crosses the membrane together with a carrier molecule.

*Production of impulses.*—According to Hodgkin (108, pp. 24, 26), metabolic reactions

are not of immediate importance in generating the spike but are essential for recovery. This is very reasonable. During the spike ions move downhill and this does not require metabolic energy—although of course it may need a complicated and tricky mechanism to change the permeability at the right time and in the right way.

Grundfest *et al.* (86) noted that sulfhydryl group inhibitors do not affect membrane or spike potentials in the squid axon, but Smith (168a) found that sulfhydryl blockade by several agents rapidly stopped conduction in axons and reduced the resting membrane potential. In agreement with Hodgkin's expectation, Keynes (123) found that metabolic inhibitors such as cyanide, dinitrophenol, and azides produce only slight changes in the spike of medullated or nonmedullated nerves. As pointed out by Spyropoulos (123, discussion), this does not mean that the generation of the spike does not involve a

chemical reaction but merely that this reaction is not dependent upon ATP or arginine phosphate. Other inhibitors of the glycolytic and TCA cycles found by Brady *et al.* (21) to have no effect on spike production include malonate, iodoacetate, potassium fluoride, fluorocitrate, and azide applied to nerve fiber and node. Previous results (141) showing that asphyxia evokes conduction block in frog's nerve trunks are ascribed by Keynes (123) to accumulation of  $K^+$  in the limited volume of external solution between nerve fibers rather than to a primary effect of lack of oxygen on the fiber membranes.

Following metabolic inhibition by dinitrophenol or cyanide, spike height decreases slightly in giant nonmedullated axons (107) and more rapidly in frog's nodes (162). In squid the decrease has been ascribed to accumulation of  $Na^+$  inside the fiber. In the node it was found, however, that spike height returns to the control value after hyperpolarization of the membrane by means of externally applied currents. On the basis of these results on nodes, Schoepfle (162) suggests that metabolic inhibitors depress spike height by increasing "sodium inactivation". According to this interpretation, oxidative metabolism would be involved in the maintenance not only of  $Na^+$  and  $K^+$  concentrations across the membrane, but also of the steady level of  $Na^+$  conductance.

Evidence that impulse activity of muscle fibers is associated with influx of  $Na^+$  and efflux of  $K^+$  was obtained by Hodgkin & Horowicz (109) in work using radioactive tracers in isolated fibers. Calculations based on measurement of fluxes and of fiber size showed that the net entry of  $Na^+$  is about  $16 \text{ pmole} \cdot \text{cm}^{-2} \cdot \text{imp}^{-1}$  and the exit of  $K^+$  is about  $10 \text{ pmole} \cdot \text{cm}^{-2} \cdot \text{imp}^{-1}$ . With a membrane capacity of  $7.5 \mu\text{f} \cdot \text{cm}^{-2}$ ,  $9 \text{ pmole} \cdot \text{cm}^{-2}$  of  $Na^+$  should enter the fiber in order to change membrane potential by 120 mv., but  $16 \text{ pmole} \cdot \text{cm}^{-2}$  are probably needed to evoke this potential displacement in the presence of increased  $K^+$  conductance. The results obtained in this research appear, therefore, to support the sodium theory as formulated by Hodgkin & Huxley (106).

Electrical activity of isolated single muscle fibers (125) and of spinal ganglion cells (124a, b) from frogs can be maintained in a sodium-free hydrazone solution, but  $Ca^{++}$  ions are essential. "An important question which remains is whether the excitatory process in sodium-free solutions is essentially identical with that in normal Ringer's solution" (125, p. 449).

Eppley (54a) has studied  $K^+$  and  $Li^+$  or  $Na^+$  transport across the membrane of the red alga *Porphyra*. He concludes that coupling between  $K^+$  and  $Na^+$  transport is not obligatory and because  $K^+$  accumulation is observed in the absence of  $Na^+$  extrusion but not vice versa, "... it seems that K uptake is the primary secretory event, with Na extrusion a secondary process dependent upon K accumulation" (54a, p. 29).

As originally noted by Overton (154),  $Li^+$  can be substituted for  $Na^+$  in the external fluid, without obvious impairment of muscle function. Keynes & Swan (123a, 124) recorded normal resting and action potentials from muscle

fibers in Li-Ringer. However, extrusion of  $\text{Li}^+$  from muscle fibers was found to be much slower than extrusion of  $\text{Na}^+$ . These findings are in agreement with previous observations by Ritchie & Straub (160), and Greengard & Straub (84) on C fibers, showing that action potentials are not altered if  $\text{Li}^+$  is substituted for  $\text{Na}^+$  except for the abolition of the hyperpolarizing afterpotentials. Apparently the "passive" transport along concentration gradients does not distinguish between  $\text{Li}^+$  and  $\text{Na}^+$  in these membranes, but the active transport is 10 to 25 times slower for  $\text{Li}^+$ .

Arthropod muscles still develop spikes when external  $\text{Na}^+$  is replaced by  $\text{Ba}^{++}$  (57). When  $\text{Ba}^{++}$  is substituted for  $\text{Na}^+$  in dorsal root ganglion cells of frogs, the spike is prolonged and eventually sustained depolarization is obtained. While the membrane is in this condition, hyperpolarizing responses may be evoked (180). Action potentials can still be obtained from B and C fibers of rabbits if  $\text{Ba}^{++}$  is substituted for  $\text{Na}^+$  in the external solutions (85). These action potentials are greatly prolonged, perhaps because of "reduction in the rate of inactivation of the sodium-carrying system" (p. 567).

Using voltage-clamp methods on nodes of Ranvier, Dodge & Frankenhaeuser (48, 49) obtained evidence that the initial current evoked by a depolarizing voltage step is carried by  $\text{Na}^+$ . The mean value for  $\text{Na}^+$  equilibrium potential with normal outside  $\text{Na}^+$  concentration was 126.3 mv. and was reduced to 105.4 mv. when outside  $\text{Na}^+$  was reduced to 37 per cent of the original value. This change is in good agreement with the change of 25 mv. expected from the Nernst relation.

Whereas in squid axons the plot of peak initial current against membrane potential gives a straight line over a wide range (104), in the frog's node the slope of the line (measuring  $\text{Na}^+$  conductance) decreased sharply for large depolarizing potentials (49). Also, the value of  $\text{Na}^+$  conductance varied considerably for two different experimental methods employed in this study. Both results indicate a form of rectification of the membrane which could be ascribed to higher membrane conductance for movement of ions from a solution of high concentration to a solution of low concentration. Goldman's "constant field equation" (81) accounted satisfactorily for this rectification and showed that the instantaneous permeability of the membrane to  $\text{Na}^+$  remained constant over a large range of potentials and was independent of external  $\text{Na}^+$  concentration.

Moore & Adelman (147) have calculated internal  $\text{Na}^+$  concentration of the squid axon by measuring the sodium potential  $V_{\text{Na}}$ , assuming that the Nernst relation remains valid. The results show a  $\text{Na}^+$  concentration of 38 mM per liter and a net sodium influx of approximately  $40 \times 10^{-12} \text{ M} \cdot \text{cm}^{-2} \text{ sec}^{-1}$  two hours after decapitation. These values are in good agreement with chemical and tracer measurements of intracellular  $\text{Na}^+$ . Internal  $\text{Na}^+$  could be increased by rapid stimulation of the unclamped axon, and  $\text{Na}^+$  could be pumped out electrically under voltage clamp by frequent pulses of the membrane to levels above the sodium potential.

Continuing previous work on voltage-clamp in squid axon (178a and

178b), Spyropoulos (170) has further analyzed the miniature responses which occur under "voltage clamp" conditions. When brief, near threshold, depolarizing pulses are applied to the axon, a threshold current response is recorded. By sampling the actual potential with a microelectrode at different positions near the center of the middle current electrode, the author has determined the presence of large-amplitude (50 mv. or more) voltage responses associated with localized parts of the membrane, the patches being approximately the same area. On increasing the depolarizing pulse further above threshold, additional steps in potential and current were recorded in a step-wise manner. This apparent nonuniformity in the membrane indicates clearly an inadequacy of the clamp. The author feels that the spatial nonuniformity is a natural property of the normal membrane.

The interpretations proposed by Tasaki and his collaborators on the basis of these and of other results have been described in a previous article (170a). Perhaps as a result of such criticisms, Cole *et al.* (35) have tested more critically their own voltage clamp requirements: (a) that the membrane potential difference be uniform over the clamped area through which the current is measured and (b) that it be constant for the duration of the current measurement. They find that their best axons give the largest current responses. Membranes that are well controlled usually produce ion currents similar to those described by Cole (34) and analyzed by Hodgkin & Huxley (106) but somewhat larger. Surface resistance of the axial electrode was found to be the most critical single factor. Their best electrodes, Cole *et al.* conclude, are marginally adequate. With axial electrode resistance slightly larger than the lowest used and with axons in very good condition, they find membrane potential is inadequately controlled and permits large differences in potential to occur over the area of the supposedly clamped membrane. They feel, however, that this type of behavior does not indicate significant nonuniformity of the membrane and is consistent with the Hodgkin & Huxley theory.

Moore & Cole (146) have further examined the resting and action potentials of the squid giant fiber *in vivo*. They find an early resting potential of 73 mv. which decays slowly to 63 mv. as a hyperpolarizing after-potential develops. They suggest that the early values may approach the normal membrane condition, thus increasing resting and spike potentials by about 12 mv. A new estimate of the liquid junction potential between 3M potassium chloride electrodes and squid axoplasm indicates a value of about 4 mv., axoplasm negative (36).

*Membrane models.*—Specific permeability at rest has been explained by Hodgkin (103, p. 387) on the assumption

that ions can only cross the membrane if their effective radii in aqueous solution are less than a certain critical value. . . . The selective properties of the active membrane are much harder to explain, since one would expect a lipid membrane to select potassium ions rather than the more heavily hydrated sodium ions.

Hodgkin suggested that the water shell of  $\text{Na}^+$  could be replaced by some other (carrier) molecule, and thus "that sodium does not cross the membrane in ionic form but enters into combination with a lipid-soluble carrier which is only free to move when the membrane is depolarized" (103, p. 388).

Mullins (150 to 152) lists a number of difficulties for the carrier system and suggests that these difficulties "can be avoided by assuming a somewhat more elaborate penetration process whereby ions move only through pores that fit them rather closely" (151, p. 1015). When the membrane is fully depolarized, pore size is distributed around a peak equal to the diameter of  $\text{K}^+$ . There will thus be high  $\text{K}^+$  permeability at zero membrane potential. With a large membrane potential, the membrane is compressed by the mechanical force exerted by the nonpenetrating ions on the two sides of the membrane, and the pores become smaller, distributing around the diameter of  $\text{Na}^+$ . Potassium permeability will then be less. Although  $\text{Na}^+$  now fits the size of the pores, it will not penetrate the membrane freely because the majority of the pores are "plugged" by  $\text{Ca}^{++}$  [ $\text{Ca}^{++}$  had already been envisioned as a "pore plug" by Gordon & Welsh (82)] since "the partition coefficient between membrane pore and solution is much greater for divalent cations than for the alkali cations" (151, p. 1015). Depolarization removes  $\text{Ca}^{++}$  from the pores and thus lets  $\text{Na}^+$  pass through the membrane. Since depolarization also removes the force which reduces pore size by compression of the membrane, the diameter of the pores will rapidly increase to a size suitable for  $\text{K}^+$  but not for  $\text{Na}^+$ .

A less elaborate theory which has many features in common with that just outlined has been proposed by Adelman & Dalton (2) to explain the action of  $\text{Ca}^{++}$  on lobster axons. According to this hypothesis, the resting membrane contains pores of about 4 Å diameter which, with normal membrane potential, are occupied by  $\text{Ca}^{++}$ , "due to forces established between the divalent calcium ions and anionic sites on the membrane macromolecules" (2, p. 617). As in Mullins' hypothesis, the pores are supposed to increase in size with decreasing membrane potential. When  $\text{Ca}^{++}$  is removed from the outside of the pores by depolarization,  $\text{Na}^+$  can rush through for a brief time. However, as the pore diameter enlarges, outward-moving  $\text{K}^+$  occupies the pores which thus become unavailable to  $\text{Na}^+$ .

Teorell (181, 182) described a model and a theory of membrane mechanisms which takes into account hydrostatic pressure in addition to the usual factors of concentration, permeability, and electrochemical gradients. The model consists of a porous membrane containing fixed charges in the pore walls and of two compartments containing solutions of different ionic composition and pressure. Experiments performed on a physical model of this type showed that "stimuli" such as electric currents through the membrane or pressure gradients may trigger oscillatory changes of membrane potential and membrane resistance. Analysis of the forces acting on the membrane to give rise to this behavior reveals a major role of electroendosmotic effects.

The author suggests that the concepts developed by analysis of these models may be of importance for interpreting the behavior of excitable cells and tissues.

#### MECHANISMS OF RECEPTOR EXCITATION

As was stated in a previous article (74), the processes normally responsible for generation of impulses in receptor organs may be subdivided into two groups: (a) specific transducer effects whereby some form of energy other than electricity evokes depolarization of sensory nerve terminals and (b) neural events whereby depolarization evokes impulse firing. The specificity of different receptor organs to different forms of energy can be ascribed to specific properties of the transducer effects, but the neural processes responsible for transforming a sustained depolarizing action into a series of nerve impulses may be essentially similar for all types of nerve cells (cf. discussion on p. 369).

The transducer action may be accomplished by the sensory nerve cell itself (for instance, in receptors possessing only free terminals) while in other cases, as in the retina, it requires activity of specialized receptor elements such as the rods and cones, external to the neurons which produce nerve impulses.

Grundfest (92) has recently proposed that the transducer action is not a specific property of receptors and has pointed out that most neurons whether sensory, motor, or interneurons, possess three functions: transducer, conductile, and effector (secretory).

Whereas in some sensory neurons the transducer processes are initiated by different forms of energy (e.g., thermal or mechanical) in other neurons (interneurons and motoneurons), they are initiated by the chemical transmitters liberated by presynaptic terminals. However, some sensory nerve cells apparently are activated by specific chemical transmitters, just as in the case of nonsensory neurons. Recent studies (76, 161) on the nerve cell in the eye of *Limulus* have been interpreted assuming that absorption of light in the photoreceptor structure leads to liberation of a chemical which evokes depolarization of the nerve cell by increasing the conductance of its membrane.

Activation of sensory nerve cells by means of chemical transmitters cannot occur in receptors possessing only free terminals, since in these the external stimulus must activate the nerve cell directly. Still, studies on the functions of nonmedullated endings of Pacinian corpuscles (117, 139) have led to the conclusion that pressure leads to increased conductance and thereby to depolarization of the membrane of these terminals. It is therefore reasonable to suppose that the mode of origin of generator potentials is in essence similar to that of excitatory synaptic potentials. Whereas most authors assume that the graded nature of generator and synaptic potentials is a basic property of receptor or subsynaptic membranes, other authors (117, 139) consider that the graded responses recorded from wide areas of a receptive

membrane originate from summation of variable numbers of all-or-none responses from very small patches of membrane.

This view, that membranes are a patchwork of small nonuniform areas, has been tentatively proposed to explain a variety of results obtained in different structures and exemplifies one of our present difficulties. In the case of pressure receptors it appears to be an unnecessary postulation. With reference to other structures, all-or-nothing behavior of patches has been discussed in previously quoted articles (35, 60, 170, 178a, b) and on p. 368 of this review.

Besides the analogy just mentioned between origins of generator and of synaptic potentials, another similarity has been noted in the features of initiation of impulses in sensory cells and in central neurons. There is now good agreement that the terminal arborizations (and sometimes the cell body) of sensory cells develop a depolarization following stimulation but do not initiate impulses. In stretch receptors (53, 55), Pacinian corpuscles (47), and visual cells (76, 184, 185), impulses appear to be initiated in the axon and, at least in some instances, they do not invade the areas in which the generator potentials are produced. These observations support the view originally proposed by Barron & Matthews (14) and Matthews (145) and more recently generalized by Grundfest (92) on the basis of evolutionary considerations, that sensory and central nerve cells possess analogous functional features.

Examples of the amazing sensitivity of several receptor organs have been mentioned by Bullock (26) in a recent review article. Among the most interesting recent studies on the functions of receptor organs are descriptions and analyses dealing with the modifications of the activity of one or more receptive units under the influence of neighboring units, of central nerve cells, of chemicals, or of different experimental procedures. The studies on visual receptors (99, 101, 129, 159), on stretch receptors (56, 128, 155), and on Pacinian corpuscles (137, 138) should be mentioned but cannot be discussed here because they exceed the field covered in this review.

#### DECREMENTAL CONDUCTION

The concept of decremental conduction was introduced 80 years ago by Szpilman & Luchsinger (175) in connection with conduction into a narcotized region of peripheral nerve. Evidence in favor of this was offered by a number of workers including Verworn (188), Lucas (143), and Adrian (3) who showed that the threshold is raised, spike height reduced, and conduction velocity slowed in narcotized nerve showing spike decrement. Kato (120), Davis *et al.* (46), and others challenged the idea of decremental conduction, suggesting that some of the experimental results were ascribable to stimulus escape, but, according to Lorente de Nó (142), these objections were removed by the experiments of Ishikawa *et al.* (116), Wiersma (190), and others. (See also 170a and 176.)

Whole nerve studies are of limited value in interpretation of single-fiber



phenomena. Uchizono has demonstrated that single nodes of frog or toad nerve treated with various narcotics show a graded spike response to varying stimulus strength (187). In normal nerve the threshold is very sharp and local responses are difficult to elicit. As narcosis develops the threshold rises, the range of stimulus strength over which a submaximal response can be obtained increases, and the amplitude of the spike decreases. These are clearly the conditions necessary for decremental conduction in either nodal or non-medullated fibers. If the spike height is stimulus-dependent over only a narrow range of stimulus strength, as in normal nerve, then activity at one node will generally be either above or below threshold for the next node; conduction will continue without decrement or else cease abruptly. In nonmedullated nerve the same narrow range of stimulus dependency would be associated with a short length of nerve showing a decrementing spike. Nerves not normally showing decremental conduction might do so when in their relatively refractory period since a wide range of local responses is easier to obtain then. It has been shown (78) that the "A" spike of the cat's spinal motoneuron is graded smoothly with strength of stimulus delivered intracellularly during the refractory period of the cell. This was interpreted as a variation in area of membrane contributing to the "A" spike, and a similar explanation could fit the graded spikes of the narcotized nerve if one postulates a patchwork of active and inactive regions.

It is generally agreed that in normal peripheral nerve, conduction is without decrement, but Lorente de N6 (142) has suggested that decremental conduction occurs normally in the central nervous system. While presenting no evidence in favor of this, he points to the advantage of decremental conduction in dendrites as a scheme for reducing the severe attenuation with electrotonic conduction. He also suggests (142, p. 597) that

in addition to subliminal synaptic potentials, . . . , to explain the integrative role of the neuron we may consider now the kind of graded responses postulated in the classical doctrine of conduction with decrement: nerve impulses of variable magnitude capable of propagating themselves through variable, but limited, lengths.

Two mechanisms are offered. (a) An impulse initiated in a dendrite which would normally die out reaches the soma if subthreshold excitatory synaptic activity also occurs along the dendrite. (b) Excitatory synaptic activity on two branches of a dendrite initiates decremental conduction in each. In either branch alone, conduction would die out shortly after entering the parent branch but, together, conduction travels further toward the soma. The suggestion that orthodromic excitation is initiated in dendrites and conducted to the soma is not supported by the evidence that spikes in cat's spinal motoneurons originate on the axon side of the soma (39, 78). But the possibility that active processes in the dendritic membrane act in a similar way to those producing decremental conduction in narcotized peripheral nerve must be seriously considered.

It has long been recognized that purely passive electrotonic spread from

the tip of a distant dendrite to the soma should suffer a severe attenuation. Rall (157) has calculated the losses to be expected on the basis of several models and points out that the proliferation of dendritic surface area because of branching may largely compensate for this attenuation. Nevertheless an assist from active processes, short of undecrementated conduction, presents an attractive addition to the hypothesis of subthreshold integration.

The degree of antidromic invasion of the dendrites following spike initiation by synaptic excitation probably varies in different cells, but it is interesting to speculate on the effect of decremental conduction on cells with extensive dendritic arborization and many dendritic synapses. Synaptically produced depolarization would be most completely wiped out over the membrane area invaded by the largest action potential while other, more remote regions of dendrites would maintain some or all of the integrated, net membrane potential resulting from synaptic activity there. Under this proposed arrangement, synapses close to the trigger area would have a commanding control over immediate impulse initiation, but their effect would last only until the next impulse. More distant synaptic activity would be integrated both in time and space and would act to regulate the general level of excitability of the neuron.

Lorente de Nó has presented good evidence for decremental conduction in narcotized nerves (142). However, he then points out that the magnitude of the spike can be affected not only by changes in concentration of external  $K^+$  and  $Na^+$  ions but also "by means of agents as varied as anoxia, alcohol, and ether, which depolarize the nerve fibers, or anaesthetics, which do not alter the value of the membrane potential." He then comes to the surprising conclusion (142, p. 598) that, "Consequently it is impossible to accept any hypothesis, such as the Hodgkin-Huxley-Katz hypothesis in which the magnitude of the nerve impulse is supposed to be directly determined by the concentration of sodium and potassium ions outside the nerve fibers." In this connection it is interesting that Huxley (115) has shown the existence of an unstable solution of the Hodgkin-Huxley (106) equations representing a small depolarization wave travelling without decrement at a much lower velocity than the usual spike.

#### SYNAPTIC PROPERTIES

Recent work on synaptic functions (the word synapse is used here to designate both the connections between nerve cells and neuro-effector junctions) is still under the influence of the important discoveries made by Katz and co-workers on the endplate and by Eccles and co-workers on monosynaptic junctions in the spinal cord. Interpretation of this work has been that, following arrival of impulses, the prejunctional terminals liberate a chemical substance which changes the conductance of the adjacent post-junctional membrane. In general, this change of conductance evokes an alteration of the membrane potential of the post-junctional cell: the chemicals liberated by excitatory nerves evoke depolarization and those liberated by

inhibitory nerves evoke hyperpolarization. Depolarization facilitates or elicits generation of impulses. Although inhibitory chemicals often hyperpolarize, they exert their action independently from potential displacement by short-circuiting the potential displacement which a given excitatory influence can induce on the post-junctional element.

The formulation of this hypothesis has led to different developments. (a) Some workers have attempted to find out what chemicals are released by different activated terminals and what postsynaptic permeability changes are evoked by their action. (b) Others have tried to establish what portions of the postsynaptic membranes are responsible for generation of synaptic potentials and what portions are responsible for generation of spikes. (c) Still other authors have challenged the whole formulation, arguing that the excitatory postsynaptic potentials are not the cause of excitation and the inhibitory potentials are not the cause of inhibition.

In order to identify a chemical substance as a natural synaptic transmitter, it would be desirable to show that this substance is released by nerve actions in amounts which reproduce the effects of nerve activity when the substance is applied directly to the postsynaptic elements. In particular the substance must be destroyed at the same rate and by the same substances that are effective with natural synaptic transmission. This difficult quantitative demonstration has been approached for acetylcholine in neuromuscular transmission (33, 126, 153) but not for other substances and junctions. However, a number of qualitative results have been collected.

Iontophoretic application of acetylcholine evokes firing of Renshaw cells (41, 45) but not of other interneurons or motoneurons. Some amino acids which are normal components of central nervous tissues (glutamic and aspartic acids) evoke delayed and long-lasting repetitive discharges of interneurons in the dorsal horns and intermediate nucleus. When applied to motoneurons they evoked some depolarization but no firing. These effects of amino acids are ascribed to "a non-specific excitatory action upon certain spinal neurones" (44, p. 612) and are not cited as proof that these drugs are normal transmitter agents. Acetylcholine could instead be the normal transmitter liberated by the collaterals of the motor axons to excite Renshaw cells. The possibility that primary sensory fibers (supposed not to be cholinergic) may synapse directly on Renshaw cells (64) raises the possibility that a cell may be excited by different transmitters.

Work on inhibitory transmitters has been inspired by the results obtained by Florey (61), Florey & McLennan (62), and Bazemore *et al.* (15) in their experiments on the inhibitory substance. It has been found that gamma-aminobutyric acid (GABA) increases membrane conductance of the crayfish stretch receptor and "clamps" the postsynaptic membrane near resting potential, just like the events following inhibitory activity (127).

Extensive studies on the inhibitory action of GABA and related compounds have been performed on crayfish stretch receptors (54) and giant motor fibers (80), on neuromuscular junctions of lobsters (93), on spinal cord

neurons (45), and on the cortex (156). In all cases GABA was the most effective compound in the series. When GABA was added to the fluid in which the stretch receptor preparation was immersed, its action decreased with time but was always restored by stirring the bathing solution. This suggests that its concentration just outside the cell decreases with time either because the drug is inactivated by the tissue or because it is taken up by the cells (54).

Gamma-aminobutyric acid in concentrations of  $10^{-7}$  gm. · cm.<sup>-3</sup> increased conductance of the membrane of lobster muscle fibers by two to ten times and decreased the endplate potential (93). As in synaptic inhibitory activity, the change of conductance because of GABA does not necessarily alter the resting potential. If the membrane potential has been altered, GABA may return it toward an equilibrium potential near the resting potential.

On spinal motoneurons, Curtis, Phillis & Watkins (45) found that GABA and beta-alanine depressed postsynaptic potentials without changing resting potential and depressed electrical excitability as measured by intracellular stimulating currents. They conclude that "these substances have a non-specific depressant action upon the whole surface membrane of neurones, both the chemically activated subsynaptic regions and the remaining electrically excited postsynaptic membrane" (45, p. 202). However, it should be noted that such depression would be expected to occur as a consequence of a conductance increase such as that demonstrated in invertebrates and does not require alterations of the spike-producing portions of the membrane.

The interpretation of the results obtained on the cortex is based on the assumption that the composite responses recorded from the surface of the cortex are made up solely of postsynaptic potentials of dendrites. Gamma-aminobutyric acid and other short-chain omega aminoacids have been found to depress excitatory synapses on dendrites while longer-chain acids evoked depression of inhibitory synapses (156). These selective actions have not been confirmed in crayfish stretch receptors (54).

There is, however, agreement in all work quoted that although the effects of GABA are similar to those of inhibitory nerve action, "there is no evidence which establishes GAB as a physiologically occurring transmitter substance" (127, p. 510).

The type of conductance change brought about by inhibitory influences has been studied by Edwards & Hagiwara (52) on the crayfish stretch receptors. The authors concluded that "the membrane potential during inhibition is mainly dominated by K<sup>+</sup>" (52, p. 315), although increased conductance to Cl<sup>-</sup> may also be involved. Similar conclusions have been reached for spinal motoneurons and are summarized in Chapter 3 of Eccles' 1957 book (51).

From the observation that strychnine and tetanus toxin decrease all forms of inhibition in the spinal cord, Curtis concludes that all these forms are likely to be "mediated by the same transmitter substance" (43, p. 191), as suggested by Eccles (51, p. 197).

An important form of progress has been the gradual realization in recent

years that nerve impulses are not the only important form of nerve activity. This point is emphasized and expanded in several recent review articles [e.g. (25, 26, 90, 91, 92)]. According to Bullock (25, p. 998), our recent "changes in viewpoint add up to a quiet but sweeping revolution."

In general, the electrical transients accompanying nerve impulses are not the cause of transmission of excitation from cell to cell [see, however (79, 119)]. Rather, a chemical liberated by presynaptic nerve activity is thought to evoke a postsynaptic depolarization by changing ionic conductance of the membrane. This depolarization (the excitatory synaptic potential—EPSP) may not evoke a further conductance change as for nerve impulses. Thus the synaptic potential differs from the spike because it is not a regenerative reaction and differs from a passive depolarization because it is accompanied, and in fact is caused, by a conductance change [see, e.g. (87)].

Because the conductance change which gives rise to synaptic potentials is neither initiated nor supported by electrical changes, Grundfest defines these responses as "electrically inexcitable" (89). This term has met with considerable (although unwritten) opposition and perhaps has somewhat obscured the gist of Grundfest's proposition.

Following an established tradition, Rall (157, p. 482) defines a membrane as passive as long as it maintains its resting electrical properties constant. A change of conductance can, therefore, be defined as activity of the membrane.

The term "excitation" has usually been employed to define production of impulses, but Grundfest uses it in a wider sense to include all forms of activity as defined above. Thus, a change of conductance brought about by a change of potential is called an "electrically excitable" phenomenon while a change of conductance which cannot be elicited by electrical changes is termed "electrically inexcitable".

If this terminology is accepted, then there should be no quarrel about the conclusion that spikes are electrically excitable phenomena and synaptic potentials are not. However, Grundfest states that certain types of nerve membrane (not certain phenomena) are electrically excitable while others are electrically inexcitable. Thus nerve fiber membranes are considered to belong to the first type and subsynaptic membranes to the second. Although this thesis is acceptable, the evidence presented to support it is not fully compelling. As long as one considers structures which develop synaptic potentials following nerve or chemical action but develop no sign of activity following electrical stimulation, the conclusion is unavoidable: these structures are "electrically inexcitable", and, presumably, "chemically excitable". Membranes which possess the ability to generate spikes are by definition "electrically excitable" since spikes are regenerative changes initiated and supported by potential changes.

The important question at this point is to determine whether electrical and chemical excitability could occur in the same membrane. There are many cells which produce synaptic potentials following nerve activity and

spikes following depolarization. In these cases one should consider the two possibilities: (a) that different areas of the cell's membrane are respectively "electrically excitable" and "chemically excitable" and (b) that the same areas have both properties. Distinction between these two interpretations becomes pointless if the areas with different properties are conceived as "intermingled" regions of vanishingly small dimensions.

*Spread of the impulse over neurons.*—With proper analysis three types of evidence can be used to determine whether parts of cell membranes do or do not change conductance because of voltage changes. Since the largest potential changes normally occurring in the nervous system are the spikes, it does not appear necessary to consider voltage changes of more than 50 to 100 mv. (a) Sign of the current through the membrane: if external electrodes are arranged so as to record an IR (current  $\times$  resistance) drop because of the transmembrane current nearby, an inward current during the rising phase of the membrane potential spike indicates activity. (b) Impedance measurements: impedance decreases markedly in excitable membranes during impulse activity. Smaller changes would occur if sizable portions of the membrane near an impaling microelectrode remained passive during the spike. (c) Recorded spike size: because of the factors mentioned in the previous paragraph, the amplitude of a recorded spike should be decreased if it is recorded from a region including a sizable proportion of inactive membrane. A decrease of resistance of the areas which do not generate impulses should further decrease the potential displacement produced by the spike. It should be noted that (a) is often technically difficult or impossible and (b) and (c) are not definitive.

These three methods have been applied recently to a variety of structures with different and sometimes contradictory results. In motoneurons the spike recorded by a microelectrode inserted in the soma presents two components (8, 9, 23, 38, 59, 78). The second phase starts only when the first component has displaced the potential by about 35 millivolts. Considerations based on spike size have led some authors to the interpretation that the first component is caused by activity of the axon hillock and the second by activity of the soma (8, 9, 23, 38, 78). Work performed recording extracellular current has led to different results and interpretations (58). Freygang & Frank (72) suggested that the giant extracellular responses indicate inactive soma membrane during the second phase of the spike [but see Woodbury (191)], whereas Fatt (58) proposed that the first component is caused by excitation of the soma and the second by excitation of the dendrites.

Finally, a recent attempt to study current voltage relations during activity has led to the suggestion that only a part of the soma-dendritic membrane is excited during the second component of the spike (66). In the eccentric cell of *Limulus*, experiments performed recording extracellular current and intracellular spikes have been interpreted assuming that the soma and dendrite of this cell are not activated during impulse activity (76, 184, 185). In crayfish stretch receptors, the results obtained by means of intra-

cellular recordings (55) would be compatible with the same interpretation, but recordings of extracellular currents (53) have been interpreted assuming that the spikes originate in the axon but are then conducted over the soma membrane.

In the toad motoneuron, Araki & Otani (10) showed that the membrane responsible for the second component of the antidromically excited spike can be fired first by direct excitation. Svaetichin (174) and Ito (118) agree that impulses can invade somata of spinal ganglion cells of frogs and toads. Following electrical stimuli applied by an intracellular microelectrode in the soma, however, Ito (118) found that the spike originates in the axon.

Hagiwara *et al.* (95), using voltage-clamp techniques on lobster heart ganglion cells, found that "the spike potential originates in the membrane of the axon some distance away from the large cell and no proper spike potential is initiated at the soma membrane" (p. 570).

The same methods applied to the large nerve cells of the mollusk *Onchidium verruculatum* (96) showed instead that spikes are produced by the soma membrane.

In the large supramedullary cells of the puffer, Bennett *et al.* (16 to 18) recorded simultaneously intracellular potentials and extracellular currents during impulse activity. They concluded that synaptically evoked impulses initiate in the axon but later invade the soma, while spikes evoked by direct stimulation of the soma initiate in the soma itself, but this may be facilitated by the simultaneous axon response. Apparently, the threshold depolarization is not very different in the soma and in the initial portion of the axon, but synaptic action is more effective on the axon because synaptic terminals are located in that region. Evidence for soma activation was also obtained in these cells by Hagiwara & Saito (94) using voltage-clamp techniques.

Discounting the rather unclear results obtained in spinal cord motoneurons and in crayfish stretch receptors, rather substantial evidence shows that the somata of (a) spinal ganglia, puffer, and *Onchidium* cells produce spikes while those of (b) *Limulus* and of lobster cardiac ganglia do not. It may not be unreasonable to correlate this difference with the observation that the cells of the first group have no synaptic or receptive areas near the soma while the others do. This inference, if acceptable, supports Grundfest's generalization that receptor and synaptic membranes are not electrically excitable.

#### CRITICISM OF THE ROLE ASCRIBED TO SYNAPTIC POTENTIALS

The sweeping revolution brought about by studies of synaptic potentials has been faced for some time by a limited but vigorous reaction. According to the views mentioned above, presynaptic activity evokes liberation of a chemical which alters conductance of the postsynaptic membrane. If the conductance change evokes depolarization, then facilitation or excitation of the postsynaptic element is caused. If, instead, a different conductance change evokes hyperpolarization (or "clamps" the membrane at a potential near resting potential), excitation is made more difficult.



The view that depolarization facilitates or evokes firing of nerve cells is hardly revolutionary. Depolarizing currents have always been used to stimulate nerves, and it has been observed in spinal motoneurons that firing occurs when a nerve action evokes a depolarizing synaptic potential of a certain magnitude or when currents evoke depolarization of the same magnitude. To many authors, the conclusion that depolarization was the cause of the firing appeared legitimate. Yet, in 1955 Lloyd & McIntyre (133) claimed to be in possession of data which made it "clear that the (excitatory) postsynaptic potential is not an essential step leading to monosynaptic reflex transmission" (p. 784). Lloyd, Hunt & McIntyre (132) suggested instead that reflex excitation is caused by the "transmitter potentiality" of presynaptic activity while facilitation of reflex responses is attributable to "facilitator potentiality" of presynaptic actions. According to the authors, these terms fill "the need for means of describing aspects of presynaptic action that are not necessarily covariant" (p. 308). The proposition that facilitation and excitation are consequences of different actions is again set forth by Lloyd (135) at the conclusion of a study of responses to repetitive stimulation. The evidence obtained there is taken to show that "there is, then, no remaining evidence to suggest that the agency for temporal summation in the monosynaptic system becomes a transmitting agency in its own right" (p. 449). In a more recent article, Lloyd & Wilson (136) state that a reflex spike can be recorded from ventral roots about 0.4 msec. before the earliest spike ever published as recorded from motoneuron soma with microelectrodes and that inhibition can be demonstrated to occur 0.1 to 0.3 msec. before initiation of the inhibitory potential. From this evidence, and from their observation that inhibitory impulses do not always evoke detectable hyperpolarization of ventral roots, they conclude that the inhibitory potential "is not an essential sign of inhibition" (p. 1226).

Thus, the criticism involves three main points: (a) excitatory postsynaptic potentials are not the cause of reflex excitation; (b) excitation and facilitation are caused by different processes, probably by different presynaptic actions; (c) reflex inhibition is not caused by the inhibitory postsynaptic potential. Some discussion of these points is presented below.

The evidence showing that reflex firing is not the result of the depolarization revealed by excitatory synaptic potential has never been produced. The only evidence relevant to this question is the observation that following asphyxia, just after the end of a phase of convulsive activity of motoneurons, reflex excitation can be elicited without detectable signs of synaptic potentials in ventral roots (131). Since firing of motoneurons is elicited by asphyxia, it is reasonable to suppose that synaptic potentials cannot be detected because the motoneurons are already depolarized to a point very near their firing level.

The claim that the reflex spike appears in ventral roots at least 0.4 msec. before it appears in the motoneuron soma is supported by a diagram (136, Fig. 2) in which a simplified response of a composite spike recorded from a

ventral root is redrawn, together with a similar sketch of spikes taken from illustrations published in articles by Eccles and his collaborators "and similar publications". The potentials recorded in these different experiments are superimposed in time by placing at time zero the start of the recorded synaptic potential. The spike recorded in one experiment from ventral roots starts about 0.1 msec. after onset of the synaptic potential, while the spike recorded in another experiment from motoneuron soma starts with a delay of about 0.5 msec. It should be noted, however, that the diagrammatic construction presented is subject to considerable error; but even if it were correct, the observation that Lloyd, recording from ventral roots, obtained firing of sacral motoneurons with a latency shorter than that which Eccles obtained recording from the soma in a lumbar motoneuron does not mean that Lloyd's spike appeared later in the soma or that Eccles' spike appeared earlier in the ventral root. Since spikes occur with different latencies in different conditions, the evidence which is wanted is to compare the timing of the same spike in the motoneuron soma and in its axon. Admittedly this requires patient experimentation, but Coombs, Curtis & Eccles (39, Figs. 1 to 3) have performed the experiment and published the results two years before Lloyd & Wilson's reconstruction. The ventral root spike always appears after the start of the spike recorded from the motoneuron soma. We have repeated and confirmed Eccles' findings after reading Lloyd & Wilson's article. In Eccles' experiments (39), the time from dorsal root shock to arrival of the reflex spike on the axon,  $t_a$ , of a motoneuron was always longer than the latency for the excitatory postsynaptic potential,  $t_0$ , in the same cell, but the time for conduction along the ventral root must be taken into account. Antidromic conduction time  $t_b$  from ventral root to cell soma is usually longer than the difference  $t_a - t_0$ . The most probable interpretation of these findings is that normal orthodromic conduction is faster than antidromic conduction because of electrotonic spread of the excitatory postsynaptic potential along the axon. But the results are not incompatible with the initiation of the spike on the axon side of the soma, perhaps in the thin segment or even at the first node.

The origin of the thesis that facilitation and excitation are different processes goes back to the time when, in order to substantiate the electrical hypothesis of transmission, it was considered important to show that the excitatory event evoked by a synchronous volley upon a motoneuron is brief. The arguments which were proposed and their criticism cannot be reviewed at this time, but some of them can be found in (7, 73, 130, 134).

The simple argument derivable from present views on transmission of excitation is that each excitatory impulse evokes a sharply rising and slowly decaying wave of depolarization of motoneurons. If impulses arrive all at the same time, these waves sum in size without much change in shape. If impulses arrive in close succession, each wave may climb from the preceding one so that depolarization may build up in time (78, Fig. 6). If depolarization evoked by synchronous or asynchronous summation reaches a certain criti-

cal level, firing occurs. Below this level, facilitation is elicited since a smaller depolarization is needed to reach the critical level.

Since the wave of depolarization evoked by a synchronous volley rises rapidly to its peak, firing level will be reached within a short time from volley impingement or not at all. Since in the absence of firing the depolarization decays slowly, facilitation will be a longer lasting phenomenon. In developing his latest argument purporting to show the different nature of facilitatory and excitatory processes, Lloyd (135) reports that monosynaptic afferent reflex volleys evoke firing which is always "linked in appropriate short latency timing with respect to afferent volleys" (p. 447). If, however, weaker monosynaptic volleys are combined with stimuli evoking (through internuncials) long-lasting excitatory activity, the intervals between monosynaptic stimuli and responses become unstable. The instability occurs equally when the monosynaptic path is stimulated at low rates (25 stimuli per min.) or at higher rates.

"Thus, not only does one find that random extraneous bombardment apparently is an essential factor in randomization of response with respect to monosynaptic afferent volleys, but also one finds that temporal summation by high frequency stimulation is not essential. There is, therefore, no reason to propose (as Alvord and Fuortes had done) that the agency responsible for temporal summation in the monosynaptic reflex pathway is capable by summation during high frequency afferent stimulation of becoming a transmitting agency in its own right" [(135, p. 448)].

The conclusions by Alvord & Fuortes (7) are thus "artificial and unjustified" (135, p. 448).

According to the view that motoneuron firing is caused by depolarization, one should expect a strict time relation between stimuli and reflex responses if there is little background activity and the stimuli evoke sharp rising large waves of depolarization. In the presence of larger background activity and smaller volleys, the firing time will be less predictable. It appears thus that Lloyd's results raise no difficulty against the view that depolarization is the cause of both facilitation and excitation (135).

Two arguments are presented by Lloyd & Wilson (136) with the purpose of showing that reflex inhibition is not caused by the inhibitory postsynaptic potential. The first argument states that inhibitory postsynaptic potentials cannot be recorded from ventral roots in normal conditions but appear if the motoneurons are depolarized. To interpret this, one need only to repeat what has been long recognized: that the important inhibitory event is a conductance change which results in a change of potential only if the membrane is not at the equilibrium potential for the ion whose conductance has changed. Inhibition is thus perfectly consistent with either a polarizing or depolarizing potential change, and no special significance is to be attached to the limiting case in which the potential change is zero. It is quite true then, and everybody agrees, that the potential shift "is not an essential sign of inhibition" (136, p. 1226); but it is quite a different matter to state that this potential shift, when it can be recorded, "may differ from the actual inhibition in

latency and time course" (136, p. 1226). The reconstruction used to prove that latency of inhibition is shorter than the latency of the inhibitory potential is the one used to show that firing occurs in ventral roots before it occurs at the motoneuron soma (136, Fig. 3). The possible imperfections of this reconstruction have been noted above, and the same arguments used there are adequate to clarify the discrepancy. The experiments of Curtis, Krnjevic & Miledi (42) confirmed by Frank & Sprague (67) showed that the IPSP does arrive later than the EPSP but pointed out that since motoneuron firing follows the start of the EPSP by up to 0.5 msec., the IPSP can begin just as the spike takes off from the EPSP and still be effective in the limiting case. In no case has inhibition been shown to occur before the arrival of an IPSP conductance change in that cell.

Both electrical constants and functional properties may be different in different parts of a cell. One surface of the cell electroplaque is completely inexcitable while the opposite (innervated) surface is probably a mixture of chemically excitable and electrically excitable areas (88). The time constant of spinal motoneurons of cats and frogs has been estimated to be between 1 and 5 msec. (9, 40, 65, 158), but in the nodal membrane of frog's axons the time constant is only 0.05 msec. (177). The part of the membrane which usually initiates impulses probably covers a small area and has properties which differ both from the properties of the soma and from those of the axon. In spinal cord motoneurons of cats (39, 78) or frogs (8), in the crayfish stretch receptor (53), and in the eccentric cell of *Limulus* (76), a depolarization sufficient to evoke excitation of the impulse-initiating region is insufficient to evoke firing of other parts of the cell membrane. In *Limulus*, the soma apparently never fires; in the supramedullary cells of the puffer, synaptic actions initiate impulses in the axon, and excitation proceeds from there to the soma (16).

At least in some neurons, the impulse-generating region may have slower accommodation (10, 65, 68) and greater tendency to repetitive firing after sustained depolarization than other parts of the cell: in spinal motoneurons, currents which depolarize the membrane in the vicinity of the soma evoke repetitive firing but the same currents applied to ventral roots do not (14, 73). In several neurons, strong depolarizing actions evoke an early burst of activity, but firing stops altogether thereafter (55, 75, 83, 87), whereas with weak depolarization sustained firing is obtained. This phenomenon has sometimes been ascribed to inactivation by excessive depolarization and suggests that only a small area of the membrane near the cell soma can respond with sustained firing to prolonged depolarization of suitable intensity. If impulses could be evoked by the long-lasting depolarization also in more distant regions of the axon, then there would be a place where the decrementing depolarization is suitable to excite but insufficient to inactivate. It is likely, therefore, that the normal repetitive firing of central neurons and receptors originates only in a localized region of the cell.

In many receptors there is little doubt that the stimulus evoking repeti-

tive firing is a smoothly sustained action. In the central nervous system the normal stimulus evoking rhythmically repetitive firing could also be a continuous action, such as would result from the combined effect upon a cell of repetitive impingement arriving asynchronously from different fibers, or rhythmical firing could be the consequence of rhythmical impingement of excitatory volleys (27, 28, 29, 63, 189). It has been suggested that rhythmical volleys of impulses could be generated by the activity of reverberating chains of interneurons, but it should be noted that Lorente de Nó (140, p. 226) originally suggested that activity of closed or open chains would probably result in continuous excitatory action.

Both in receptors and in spinal motoneurons, the effects of a steadily sustained natural stimulus are duplicated by a steady depolarizing current. The properties of these artificially evoked responses have been recently studied in many cells: invertebrate axons (1, 11, 12, 102), eccentric cells of *Limulus* (75, 76, 97, 144), the crayfish stretch receptor (183), cerebellar neurons of frogs (173), supramedullary neurons of the puffer (17, 94), and spinal cord motoneurons of cats (77). In many cases it was found that frequency of firing is a linear function of the intensity of the depolarizing current [see also (122)], but it is well to keep in mind that the approximate linearity of this relation may well be the result of combination of several nonlinear processes and almost certainly does not reveal the existence of a single and simple factor governing frequency of firing.

Concerning the mechanisms giving rise to rhythmical firing following a steady depolarizing action, Adrian (4, 5) suggested that rhythmicity of receptor cells is controlled by refractoriness: a prolonged stimulus evokes an impulse at the onset (just as a transient stimulus would do) but a following impulse cannot be discharged until the structure has recovered from the aftereffects of the preceding firing. This interpretation implies that, with a stimulus having the shape of a step function, the latency for discharge of the first impulse should be considerably shorter than the intervals between following impulses.

Arvanitaki (11, 12) and Hodgkin (102) found, however, that when certain crab's nerves are stimulated by depolarizing current steps, the latency for the first impulse is approximately equal to the interval between first and second impulses. Hodgkin also determined the course of recovery from single firing and found that frequency of firing is often much slower than refractoriness would justify. He deduced that frequency of firing of these nerves is primarily controlled by the time required to displace membrane potential to threshold value and suggested that this "excitation time" is controlled in turn by slow development of a local response. This interpretation is in agreement with a previous conclusion by Eccles & Sherrington (50) that slow development of a "central excitatory state" rather than refractoriness controls the normal repetitive reflex firing of spinal motoneurons. Hodgkin's interpretation (102) cannot be applied to the majority of cells mentioned above because it was found that the latency for the first impulse is usually con-

siderably shorter than the intervals between successive spikes. This suggests that refractoriness is an important factor controlling frequency of firing. However, one should keep in mind the possibility that in addition a depressant action of the sustained depolarizing current [inactivation; see (105)] also may increase progressively in time, causing a decrease of frequency (adaptation).

Even if different processes exert a predominant role in different cells to control frequency of firing, in all cases mentioned rhythmical firing is evoked by a sustained stimulus because of the periodicity of the events occurring in the firing cell itself. For instance, in the crab's axons (12, 102), the stimulus produces progressive increase of depolarization to firing level, and the impulse destroys the depolarization so that the slow depolarizing process has to be started over again after each impulse.

In certain cases, repetitive responses of neurons in the central nervous system are a consequence of repetitive impingement of afferent volleys. This is obviously the case when repetitive stimulation is purposely applied, but it may also happen that a nonperiodic stimulus is transformed into a rhythmical train of volleys by virtue of the interconnections among neurons.

Rhythmicity of discharge of cells in the brain or brainstem has been interpreted as a result of repetitive impingement of volleys generated in reverberating chains of interneurons (27 to 29). The evidence for this contention is that a massive electric shock which presumably excites simultaneously all neurons in a large area disrupts the rhythmical activity. This effect is ascribed to inactivation by refractoriness of closed internuncial chains.

Wall (189) concludes that repetitive firing of spinal interneurons in the dorsal horns of cats is caused by repetitive activity originating outside the cell itself on the basis of experiments performed with intracellular electrodes. A train of spikes was recorded from certain dorsal horn interneurons after a single shock to a peripheral nerve, and the timing of each impulse in the train relative to the stimulus remained constant in different trials so that if the stimuli were aligned all spikes were also aligned in superimposed records. When the same orthodromic stimulus was followed at an appropriate time by a stimulus evoking firing only of the impaled cell, the timing of the early impulses in the repetitive response was altered but the later impulses originated at the same time as when no additional stimulus was interjected. Wall concludes from this that "the time of the rhythm is not reset by an interjected stimulus" (189, p. 318). It would appear more appropriate to say that the time of firing of the repetitive impulses was altered at the beginning but not at the end of the train. Since the evidence presented shows the effect of an interjected stimulus delivered only at one particular time relative to the orthodromic stimulus, the results could be interpreted by assuming that the interjected stimulus in fact produced resetting but also slowed down frequency of firing of the first few impulses. In similar experiments performed with dorsal root reflex discharges, Wall (189) showed, in fact, that an interjected stimulus may fail to show resetting if delivered at a certain time (his

Fig. 3) but evokes clear resetting if delivered at a different time (his Fig. 4).

In the first of the two main mechanisms mentioned so far, periodicity is controlled by the firing of the cell, while in the second it is controlled by external influences. There is still another mechanism in which rhythmicity is primarily controlled by events other than firing which, however, occur in the cell itself. Rhythmical oscillations of potential occur in certain cells in the absence of firing. If these attain sufficient amplitude, impulses may originate on the depolarizing peaks but frequency is not necessarily affected very much by the firing itself. This phenomenon was demonstrated several years ago (13, 22) in peripheral nerves deprived of  $\text{Ca}^{++}$ . More recently, Huxley (114) calculated the features of oscillatory responses in low  $\text{Ca}^{++}$  on the basis of the Hodgkin & Huxley formulation. Oscillations resulting from the action of depolarizing currents have been mentioned as occurring in other structures (17, 75, 94, 102). Bullock & Terzuolo (24) have found evidence of rhythmicity independent from impulse discharge in certain heart ganglia of invertebrates. The rhythmical activity of these cells is present in the absence of applied stimulation, and in certain cells it may originate in more than one locus, giving rise to complicated rhythms.



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# SOMATIC FUNCTIONS OF THE NERVOUS SYSTEM<sup>1,2</sup>

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## INTRODUCTION

This review represents an attempt to bring together recent data from the wide and many-faceted field of somatic functions in the nervous system. In regard to the impressive number of available documents pertaining to this topic, this report is undoubtedly incomplete and overschematized; because of space limitation, many outstanding papers had to be eliminated and many others summarized too briefly in spite of their high value. Selection reflects only the authors' own interest and tendencies. Following previous examples, presented material has been grouped into sections devoted to the usual anatomofunctional entities.

## BOOKS AND MONOGRAPHS OF GENERAL INTEREST

This year, as every year, books have appeared which summarize or develop papers and discussions presented at symposia, meetings, or interdisciplinary conferences. Among those dealing with subjects which partly, at least, concern our present topic, the following should be mentioned: *First International Congress on Neuropsychopharmacology* (56); *Symposium on Curare and Curare-like Agents* (54); *Les grandes activités du lobe occipital* (8); and *Actualités neurophysiologiques* (314). The first volume of a series of *International Reviews of Neurobiology* also contains some interesting papers on neurophysiological problems (352).

The impressive number of papers presented at the First International Congress of Neurological Sciences (Brussels 1957) has been published (51). *The Proceedings of the XXII International Physiological Congress* in Buenos Aires report on some symposia dealing with functions in the central nervous system.

New books of general interest to students of the central nervous system are also available. Special reference must be made to the first two volumes of the *Handbook of Physiology* devoted to neurophysiology, of which Magoun was the Section Editor. They present many excellent articles concerning the

<sup>1</sup> This review is based upon publications which were available to the authors for the period extending through June 1, 1960.

<sup>2</sup> The following abbreviations are used in this chapter: DC (direct current); ERG (electroretinography).

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functions of the central nervous system; separate chapters will be mentioned later (291). Schaltenbrand & Bailey's *Introduction to Stereotaxis* (382), which is an extensive photographic presentation of the macro- and microscopic anatomy of the human brain, also presents contributions of general interest in the fields of functional neuroanatomy and neurophysiology. Extensive anatomical monographs, clearly helpful for present or future physiological work, are available on the dog's brain (5), the diencephalon of *Talpa* (34), and the dolphin (262). A new edition of the well documented *Cerebral Cortex* by Ajurriaguerra & Hécaen (7) has appeared. Penfield (349) briefly summarized his observations on cortical stimulation in man. Volumes more specifically concerned with a particular theme will be referred to in the corresponding sections. Other documents in book form dealing with physiological processes involved in conditioned reflexes [Anokhin (22); Asratyan (29)], or mechanisms of learning and behavior [Brazier (58)], will be reviewed in the following chapter in this volume.

### SPECIFIC AFFERENT SYSTEMS

#### THE SOMATIC SYSTEM (INCLUDING PAIN)

*Cutaneous receptors.*—Mechanisms of cold and warm reception were reviewed by Zottermann (291). Cold fibers, but no warm fibers, have been found in nerves from the chicken and pigeon tongue (249) and from the skin of the cat limb; the latter fibers were also sensitive to mechanical pressure (441). On the other hand, specific mechanical receptors, not reacting to thermal stimuli, were identified from the infraorbital nerve of the cat and rat (53). Discharges in response to mechanical stimulation of the vibrissae of the cat were described (243), and responses of dental nerve endings to mechanical, thermal, and electrical stimuli were considered in the dog (428). According to Wall (429), pressure-sensitive A fibers from the skin form a continuous group of varying diameters; spinal cells in the dorsal horn respond to various modalities of skin sensation with differences in discharge patterns depending on the nature of the stimulus. There is a growing interest in a signaling role of C fibers for nonpainful stimuli. The cat saphenous thus contains unmyelinated afferents sensitive to moderate cooling; responsiveness to warming or to mechanical stimuli remains moot (129, 225). Bing (45) observed that various drugs (acetylcholine, atropine, histamine) applied to the human skin could either increase or decrease the proportion of cold spots to pain spots.

Receptors sensitive to cutaneous temperature below 25°C., presumably concerned with cold pain, were identified from the infraorbital nerve in rats (52). From a more general point of view, problems in nervous mechanisms for pain were outlined in several books and reports (97, 338, 446).

*Muscular and deep receptors.*—A well-documented paper by Gray (192) summarized the available data on three types of mechanoreceptors (crustacean stretch receptor, frog muscle spindle, and Pacinian corpuscle), with emphasis on their common transducer properties.



In the rabbit, three types of spindles could be distinguished on the basis of their rate of discharge (slow, medium, and fast). Fast spindles (mostly found in phasic muscles) respond better to fast extensions, and medium and slow ones (in tonic muscles) better to slow rates of stretch (186, 187).

Responses from tension receptors of the medial collateral ligament of the knee joint were recorded in the cat (21, 398). It has been clearly indicated by Mountcastle & Powell (327) that joint movement is the adequate stimulus for certain somatic neurons, which are unaffected by skin or deep tissue stimulation.

*Central somatic pathways.*—A comprehensive article by Rose & Mountcastle (in 291) summarizes data on central organization for touch and kinesthetic reception.

It has been confirmed that the lateral divisions of the dorsal spinal roots preferentially convey pain (397). In the cat, the afferent pathway for tactile placing reaction of the hindleg was located in the dorsal part of the lateral funiculus (283). A new group of fibers was isolated in the dorsal spinocerebellar tract, which could be activated by both II and III afferents of muscular and cutaneous origin (284). Somatic responses in anterolateral spinal tract were found to be more susceptible to anaesthetics than those in dorsal column (365).

Several groups of results deal with somatic projections into "nonspecific" structures. In the cat, the C fibers activate the ventral midbrain tegmentum (102); "pain" impulses possibly pass through the medioventral tegmentum and not through ventral thalamus (397), while responses to sciatic A fibers stimulation are described in the hypothalamus (150, 151). Short-latency responses to somatic stimuli of various loci were recorded from cells in both the small and large cell divisions of the red nucleus. In the latter division a late activation, moreover, is caused by cerebellar reverberation (302). Absence of somatotopy was also recognized in responses recorded in the centro-median nucleus of the monkey under chloralose (12) or of the awake cat (9, 11). Also anatomists (309) have followed fibers in the monkey through the anterior and lateral spinal funiculi to the lower reticular formation, central gray, and intralaminar thalamic nuclei. In the cat, the diencephalic distribution of spinothalamic fibers was similarly analyzed (19).

There is a growing interest in the study of somesthetic projections into the posterior nuclear complex of the thalamus. Responses obtained therein to stimulation of cutaneous nerves (437) or of skin and deep receptors (354) show no clear somatotopical organization; this complex appears to be an essential relay for the spinothalamic route (354). Neurons from the lateral as well as medial division of the ventroposterior thalamic nucleus (VPL and VPM) are distributed to somatic I area, others to somatic II, and some to both as shown by anatomical studies (287). Among projections from the tongue, which were identified in the ventroposterior median thalamic nucleus of the cat, some neurons responded only to touch, others specifically to cold; the tongue receptive field appeared distinct from that of the face (269, 270). Somatic cortical responses were systematically mapped in the raccoon (435).

and their limits outlined in the chimpanzee (208). The pattern of increase of response in somatic I area caused by graded electrocutaneous stimulation in the cat was described (374), and cortical responses to stimulation of vibrissae were also mapped and characterized (243). Results by Mountcastle & Powell (358, 359) provide essential data on the functional organization of the primary somatic system in the monkey: the three distinct 2, 3, 1 architectonic fields differ significantly because of the dominant reactivity of their elements either to cutaneous or deep tissue stimulations. Central inhibitory interactions restrict the size of the cortical receptive field in such a way that cells, excited by one spatially located stimulus, become surrounded by a ring of cells which are inhibited by the same stimulus (328).

Various observations related to problems in somesthesia have been made on conscious human subjects, such as recording thalamic ventral-posterior responses to tactile stimulations and observing their variations in amplitude when attention is directed toward one or another sensory modality (231), evaluating with quantitative scores permanent losses in somatosensory functions following extensive cortical wounds in war veterans (385), and detecting significant spatial summation effects between two separate cutaneous pain foci (36).

#### THE GUSTATORY SYSTEM

Pfaffmann (in 29) clearly summarized the essential known data on the mechanism of taste, and Zotterman (445) also, reviewing results from his group and others, especially considered the problem of limits in specificity of the receptor responses. The old notion of four fundamental qualities (sweet, sour, bitter, and salty) has been roughly confirmed by electrophysiologists in the cat, rat, bird (249), frog, and recently monkey (182), and even man though some receptors respond to two or more modalities. Unexpectedly, the existence of receptors responding to pure water (447) has been demonstrated in the cat and monkey, but not in the rat and man.

In the albino rat, a cortical gustatory area and a gustatory thalamic relay were electrically identified by stimulating the ninth nerve and chorda tympani; bilateral ablation of this cortical zone caused severe disturbances of taste discrimination (37).

#### ORGANIZATION OF VISUAL PATHWAYS AND CENTRAL MECHANISMS

There is a renewed interest in studying optic centers in lower vertebrates. Evidence was given that after regeneration of the cut optic nerve in *Xenopus* (166) and frog (308), fibers reintegrate their appropriate places in the tectum. Dorsal midbrain ("tectal") responses elicited by electrical stimulation of the optic nerve, or by different kinds of photic stimulation (white or colored lights), have been described and their significance discussed in various fishes (255, 256, 323), in the toad (93), and in the chicken (106). Moreover, optic stimulation facilitates responsiveness of the vestibular system in the toad (95).

Some data in mammals concern subcortical "accessory" centers. Anatomists have considered optic projections to the mesencephalic tegmentum in the cat (204) and to the brainstem in the mouse (368). In the latter species, cytoarchitectonic organization of the pretectal region was described (391).

Patterns of unitary responses to light were analyzed in the pretectal region of the cat (337). Suggestions were made that in the cat the superior colliculus might be involved in more complex operations than is usually believed, particularly with visual (50) or intermodal (visual, tactile, acoustic) integrations (390).

Optic projections to the cerebellum were considered, with particular reference to the effect of anaesthetics (147). Studies in the lateral geniculate body of the cat allowed conclusions on the recovery cycle of its synapses (49), on interactions between projections from the two eyes (46), and on changes in presynaptic responses following optic nerve tetanization (47) it was also shown that geniculate neurons may receive projections from non-optic sources, mainly from the reticular formation (24, 81).

Of physiological interest are the degeneration studies concerning patterns of endings of optic axons on the geniculate cells in the cat (203) and the chronology of transneuronal changes in geniculate body of the monkey (305).

Available reviews on the cortex report on the anatomy of the occipital lobe (112), electrophysiology of the visual area [(79); Bartley in (291)], and on correlations between the latter data and psychophysiological phenomena in man (236, 237). Visual cortical responses were explored in the newborn kitten, and the changes were considered to be associated with maturation up to the twentieth day (298, 299). The evoked potentials to light were mapped in the chimpanzee (208) and representations made of the visual field in the baboon and monkey, subject to a quantitative evaluation of a "magnification factor" (mm. in cortex per degree of visual field) (119).

Through unitary recordings, receptive fields of single neurons in striate cortex of the cat were mapped (215) and differential effects of ipsilateral or contralateral eye stimulation on one cortex detailed (77). Using his procedure for single-cell recording on unrestrained cats, Hubel (213) added new data on patterns of firing of cortical neurons, some cells appearing to have a particular sensitivity to a moving light source. A similar method more recently allowed subcortical recording of unitary response patterns from optic tract, thalamus, and radiations; and differences from those of the cortex were thus stressed (214).

It was further demonstrated in the cat (110) that critical flicker frequency of cortical isolated neurons may be influenced by stimulation of unspecific thalamic nuclei. Vestibular influences were also shown to act on cells in the visual cortex. This result might provide a possible basis for co-ordination of vestibular and visual afferent influences (194 to 196).

Late cortical responses to flash, observed during deep barbiturate anesthesia, were compared to Forbes's "secondary discharges" obtained under the same conditions for sciatic nerve stimulation (111, 154).

Variations in amplitude of optic responses were observed at various stations during experimental anoxia in the cat (152) and under drugs such as lysergic acid diethylamide or serotonin (48, 251, 252). Other investigations on cats report variations during arousal (332), and, in animals with implanted electrodes, during habituation (293 to 295) as well as in relation to visual attention (212). Arduini & Hirao (26) attributed the "Chang effect" in the cat (facilitation of cortical responses to geniculate stimulation by a steady illumination of the eye) to a release, during illumination, of an inhibition exerted on higher centres by the retina when kept in the dark. The size of responses recorded from the human scalp by instrumental integration was also shown to depend on the degree of electrocortical arousal (85), and evoked potentials from optic radiations in conscious subjects varied significantly, depending upon the type of attention or of subjective perception (205, 235). In the monkey, critical flicker frequency was impaired both by lateral occipital and inferotemporal lesions (312).

#### THE AUDITORY SYSTEM

*Receptors and lower central levels.*—A volume by von Békésy (35) outlines the essential knowledge on peripheral processes (conduction of sound, cochlear mechanisms, etc.). A discussion on "hearing under water" was summarized in three papers concerning hearing in bony fishes (126), aspects of mammalian hearing under water (369), and the function of the cetacean ear (159).

Microelectrode studies on barbitalized cats have clearly shown that all three major divisions of the cochlear nucleus are "tonotopically" organized, each one being likely to command a full tonal spectrum (372), and that in the superior olivary complex, a class of units exists concerned chiefly with preserving exact time of arrival of stimuli at the cochleae, and therefore presumably devoted to sound localization (162).

*Upper central levels.*—Problems of cortical projections in the cat [Ades, in (291)] continue to be considered. Afferents to acoustic area I and area II AII-Ep) are independent, but close corticocortical connections exist between both neighboring cortical fields, as demonstrated carefully by Downman *et al.* (130). A low temporo-orbital focus responding to clicks, independent of the already known acoustic areas I, II and III, was also identified and termed area IV (125). Cortical acoustic responses in the chimpanzee have been described (208). Precise data on the cerebello-cortical connections concerned with auditory projections were also obtained (399). In unanesthetized cats, cortical responses to repetitive clicks could be followed through instrumental integration up to 200 clicks per second (180). The shapes of evoked potentials aroused by near-threshold sound pulses, as well as the influence of spontaneous electrical activity on responses to clicks, were evaluated statistically on narcotized dogs (418, 420). Behavior of single units from cortical acoustic areas I and II of the cat, or from various other levels of the brain, were com-

pared; a general conclusion was drawn that pitch and intensity discriminations might be mediated at the thalamic level, and timbres perceived at the cortex (240, 241, 433). In fact, wide-frequency curves were also obtained from cortical single units in normal unrestrained cats (239).

Much other new information on auditory functions has been obtained by electrophysiological or behavioral studies: localization of sounds (101, 244, 371), and discrimination of frequencies (412a) and of tone patterns (336). Since an extensive chapter in this volume is devoted to problems of audition, there will be no further discussion of these points here.

#### THE VESTIBULAR SYSTEM

Although electrophysiological data have also provided most of the recent findings in this field [see the review by Gernandt (in 291)], nevertheless some investigations on vestibular functions have been performed by use of ablation methods and behavioral observations. Blind octopuses (55) are not completely disorientated by bilateral ablation of statocysts, a fact suggesting the role of other sensory systems in equilibration. Gribenski (193) developed a method for and studied the effects of grafting the nerve branch innervating one horizontal semicircular canal to the anterior vertical ampulla in the frog. A marked decrease in postural tone, resulting from ipsilateral destruction of utricular macula (143), was observed in the pigeon. Effects of specific partial labyrinthectomy were described in the kangaroo rat (229). The monkey's ability to compensate for vestibular disturbances was shown to be greatly impaired by pre-existing cerebellar ataxic deficits (342).

In the statocyst of *Homarus* (100), the rate of afferent discharges appeared to be a function of static hair deflection. In the cat, single units in the bulbar medial reticular formation were shown to be rhythmically activated (132) during vestibular nystagmus; vestibular excitatory or inhibitory influences upon cortical visual neurons were also detected (195).

With regard to extrinsic influences upon vestibular neurons, projections from the anterior lobe of the cerebellum to Deiter's nucleus were mapped physiologically (357). The descending vestibulospinal activity was found to be influenced by the head position and inhibited by cerebellum and, independently, by reticular formation (170). Complex two-way interconnections between the fastigial nucleus, the labyrinth, and the vestibular nucleus were also described anatomically (87).

#### THE OLFACTORY SYSTEM

Essential data on the olfactory system, receptors, and processes involved in olfactory stimulation, as well as central connections, have been summarized by Adey (in 291). Some new facts or interpretations concern slow potential waves recorded in the rabbit or frog from the nasal mucosa and olfactory bulb in response to stimulation by odorized air; these appear to be involved in the initiation and transmission of olfactory signals (289, 343 to 345). On

the other hand, interconnection between olfactory bulbs has been confirmed electrophysiologically (430). As postulated by Hernández-Péon *et al.* (206) the olfactory bulb is submitted to a centrifugal influence coming from the reticular formation (see below).

## REFLEX ACTIVITY OF THE SPINAL CORD

### REFLEX PATTERNS AND SPINAL ORGANIZATION

Reviews on functional patterns of connections in the spinal cord (134, 184, 282) are available, as well as a general outline of spinal mechanisms involved in somatic activity [Lloyd (in 291)].

It was postulated by Szentágothai from a degeneration study in small neurally isolated portions of the spinal cord (404) that excitatory and inhibitory fibers may have distinct modes of endings on motoneurons.

Electromyographic studies by Hagbarth (201) on nociceptive skin reflexes of the lower part of the legs of normal human subjects have revealed that, besides the usual flexion reflexes, local extension reactions may be elicited from skin areas covering extensors. From a comparison of various species, it appeared that their type of gait and the dominant pattern of their crossed reflex (symmetry or antagonism) may be correlated (38).

Forelimb extensor rigidity, observed in the decerebrate Hawaiian toad, is caused by the hyperactivity of the small nerve motor system, maintained by afferent influx from skin receptors and proprioceptors (92). Following adequate stimulation of ligamentary receptors, joint-protecting ligamento-muscular reflexes were observed neither in the decerebrate cat nor in man (21, 351, 398). Repetitive stimulation of I and II muscle afferents was shown to produce an increase in pulmonary ventilation; cutaneous stimulation, in contrast, was ineffective (44).

Refined and systematic data are available on synaptic facilitatory or inhibitory actions of Ib, II, and III muscle afferents, or joint afferents, on distinct functional groups of alpha motoneurons of the spinal cat (137, 271). It was also demonstrated that myotatic and inverse myotatic reflexes are associated with reciprocal changes in motoneurons innervating corresponding muscles of the contralateral hindlimb (350). Recurrent Renshaw effects in the spinal cord show an organized distribution of their inhibitory or facilitatory action related in some respect to reciprocal innervation (439). Recurrent inhibition may have a functional role in limiting stretch reflexes to the path actually stimulated (65).

### SPINAL MECHANISMS

Distinct patterns of motoneuron discharges in the frog were evoked by stimulating either the ipsilateral dorsal root or ipsilateral lateral column (64). Observed differences may depend, as further shown (63), upon sites of excitation of the activated neurons (either distal dendrites or proximal). In the same animal it was also confirmed that, in contrast to cutaneous afferents,

muscular afferents mainly activate ventral structures of the spinal cord (300, 301).

In the decerebrate cat, the active myotatic tension developed in postural muscles was shown to be a linear function of extension (306; see also 184, 185); this constant "loop gain" is unaffected by gamma paralysis (307), but is augmented (356) in the forelimb under conditions "releasing" alpha motoneurons, such as postbrachial section of the cord, deafferentation of the contralateral forelimb, and cerebellectomy.

Properties and functional importance of interneurons continue to be analyzed; micropipette recordings allowed a comparison of their electrical properties with those of motoneurons (220) and revealed their tendency to possess a tonic background repetitive activity and to respond with repetitive firing to a single afferent volley (221). The presence of an inhibitory interneuron in "direct" spinal inhibitory pathways, as previously postulated by Eccles, is still discussed. By comparing the time courses of postsynaptic potentials in motoneurons with the time course of direct inhibition, this possibility has again been supported by Frank & Sprague (157) but challenged by Lloyd & Wilson (279) [see also Lloyd in (291)]. Functional failure of interneurons might explain the chronic ischemic rigidity in hindlimbs (167); strychnine tetanus could likewise be caused by circuit activity involving them (227). In discussing spinal shock mechanisms (410), it was also suggested that it is principally the interneurons which are disturbed by cord transection.

Another line of interest concerns enduring modifications in spinal excitability. Modifications of postsynaptic potentials of motoneurons evoked by monosynaptic repetitive stimulation at various frequencies were considered (116), and an increase in presynaptic spike height was shown to be at least partially responsible for the posttetanic potentiation of synaptic transmission (135). After chronic spinal section at the thoracic level, lower limb monosynaptic reflex responses usually show a repetitive efferent firing in response to a single afferent volley (401). After chronic severance of the afferent paths from a given muscle, the corresponding motoneurons showed depressed excitatory postsynaptic potentials and increased posttetanic potentiation in response to stimulation of the severed nerve (136); the suggested interpretation of these results has been questioned (39). Repetitive spiking recorded from the lumbosacral ventral root in a cord completely deafferented and transected at I1 has been attributed to gamma activity (278).

*External factors and pharmacological actions.*—Koizumi *et al.* summarized the excitatory effects of cooling on spinal reflex action in mammals (253). The patellar reflex may be augmented or depressed by stimulation of the sciatic nerve, depending on experimental conditions (frequency of stimulation, anaesthetics, etc.) (379).

Pharmacological studies also include effects of curarizing drugs (389), of central depressants (440), of tetraethylammonium (432), a comparison be-



tween actions of tubocurarine and cardiazol (115), and arguments that the depressant action of  $\gamma$ -aminobutyric acid [see (141)] and the excitatory action of certain other aminoacids on motoneurons are nonspecific and unrelated to a particular type of transmitter action (117, 118).

### MOTOR MECHANISMS

Documented articles are available on various aspects of motor mechanisms, such as the pyramidal system [Patton & Amassian, in (291)]; extra-pyramidal mechanisms [Jung & Hassler, in (291); Bucy, in (382); Schaltenbrand & Hufschmidt in (382)]; and sensorimotor integration [Terzuolo & Arden, in (291)].

*Pyramidal system.*—Differences in distribution of descending fibers from the precentral and postcentral cortex to the lower brainstem and spinal cord were studied anatomically in the monkey (267).

Antidromic stimulation of the medullary pyramids in the cat, known to produce a two-wave cortical response, gave only a single component when the tract had been previously isolated from the bulb (416). By studying peripheral discharges in response to stimulation of the motor cortex, inhibitory interactions between both hemispheres were noticed (245). The correlation, often questioned, between the Babinski sign (which is considered as part of the pathological general nociceptive flexion reaction) and pyramidal tract dysfunctions has again been discussed (57) and again regarded as likely (268).

*Non-pyramidal cortex.*—"Supplementary" (in the sense of non-pyramidal) motor areas in the primate participate in complex and associated movements (114, 384). Stimulating the occipital lobes also produces complex motor responses, as detailed by Rieck (370). Patterns of eye deviations in response to cortical stimulation were again considered in the macaque (113) and described for the frontal eye field in man (274).

Stimulation experiments in unanaesthetized cats revealed a motor representation on the supracallosal mesial cortex, somatotopically arranged in two distinct funiculi (217). In the macaque also, areas 23 and 24 both possess a somatotopic motor localization from head to tail, but in reverse order for each (393). In the same animal, anatomical studies have also indicated that motor areas of the mesial frontal cortex project upon various other cortical (areas 4, 6, and 8) as well as subcortical structures, such as the reticular formation and the centromedian nucleus (426). From a study of the grasp reflex in the monkey, following frontal lesions (including areas 6, 8, and cingulate), the conclusion was drawn that the cutaneous (and not the proprioceptive) stimulus acts as the essential initiator (376).

*Subcortical mechanisms.*—Anatomical (230) or electroanatomical data (275, 421) are available on connections in both directions between basal ganglia and other central structures.

In connection with the puzzling problem of the function of basal ganglia, data on cats indicated a suppressive effect of caudate stimulation upon

seizure phenomena developing elsewhere in the central nervous system (421) and a facilitation, by pallidal stimulation, of the tremor induced by stimulation of the midbrain tegmentum (13, 14). On the other hand, sensory projections of different modalities were again identified in the caudate nucleus of the cat under chloralose (11a).

Impulses in the monkey responsible for dyskinesia caused by subthalamic lesions appear to be selectively mediated by the lateral corticospinal tract, a fact suggesting complex relationship between pyramidal and "extrapyramidal" systems (88). In the cat the subthalamus (3) and pallidum (2) were both found to act upon reticular neurons.

Ascending and descending efferents from the red nucleus to the spinal cord, cerebellum, and thalamus were identified anatomically (207). Patterns of eye movements in response to stimulation of the tectum and tegmentum in the cat were checked, and the final goal position shown to depend upon the stimulated point (224). Voluntary saccadic eye movements in man and their velocity curves were also considered (223); they are distinct from conjugate eye movements in the cat evoked by brainstem stimulation (222), but their characteristics resemble those of human optokinetic nystagmus, a fact which could suggest a common physiological mechanism for both phenomena (378). Small lesions to the monkey's brainstem, which produce defects in vestibular nystagmus, affect optokinetic nystagmus as well (412a). Vestibulo-ocular reflexes and nystagmus in response to central stimulation in rabbits may also have, at least partially, a common mesencephalic substrate (40). In connection with these experimental data, Kornhuber (257) indicated that human nystagmus alternans may be caused by bilateral lesions in the pontomesencephalic tegmentum.

Cats with electrolytic lesions of periaqueductal gray grew mute and also stopped purring, though ability to express satisfaction otherwise was left intact (1).

*Motor unit; muscular activities.*—Conduction velocities in normal human motor nerves to various small muscles of the hand and the foot were measured and compared (412). A restimulation of motor endings by muscle action potentials themselves was found likely to occur during a muscle twitch, thus making some fibers contract tetanically (67). A group of interesting new data concerns the functional distinction between red (tonic) and pale (phasic) muscles and their corresponding motoneurons. Kuno (264) concluded that, with respect to their afterpolarization and antidromic inhibition, motoneurons show a continuous gradation between extreme types rather than a clear separation into two categories. According to Buller *et al.* (72, 73), all muscles are slow in the newborn cat and later become fast unless their "slow properties" are maintained through control from the spinal cord; an unknown substance rather than nerve impulses as such might underlie this neural influence.

Other new facts relate to muscle spindle innervation. Mammalian intrafusal potentials in response to stimulation of gamma efferents were shown to

be typical propagated activities (144) which remain constant in amplitude during a repetitive gamma stimulation facilitating sensory spindle discharges (145).

In sheep or goats, the oculomotor nerves contain numerous small motor fibers (127); those from the fourth nerve could be stimulated in isolation, thus eliciting an increased sensitivity of extraocular muscle spindles without any increase in muscle tension (438). The importance of proprioceptive control in eye fixation has been emphasized for man (199).

Some events in the muscle spindle were recently shown not to depend upon gamma innervation, such as prolonged changes in spindle sensitivity of an extensor muscle following a tendon tap or a twitch of the muscle itself (188), and also short-latency spindle discharges which may accompany a muscle contraction in response to stimulation of its motor path and possibly reveal an alpha activation of spindles (189, 190).

Buchthal & Sten-Knudsen (71) reviewed the problem of impulse propagation in striated muscles. A physiological method was developed, based on multilead recording, for outlining diameters of motor unit territories (70). Differences between muscles were interpreted in terms of differences in fiber concentration and total number of fibers per motor unit. Problems in the physiopathology of neuromuscular transmission were considered (98, 99, 123); muscular biopsies on myasthenic patients revealed two varieties of abnormal motor endplates, one seeming specific to the disease.

In the decerebrate rabbit or cat the tonic activity existing in intercostal muscles indicates that, apart from their respiratory function, they also play a postural role (304). The mechanism of talking under various conditions (131) was analyzed by recording esophageal pressure, volume of air in the lungs, and electrical activity of respiratory muscles. Electromyography from laryngeal muscles suggested to Buchthal (69) additional arguments for a "myogenic" theory of pitch, according to which frequency of sound is determined by the tension of intrinsic laryngeal muscles.

### CEREBELLUM

A histological study suggested that most of the climbing fibers in the cat's cerebellar cortex are derived from the olivary complex (405).

From differences in actions of drugs, it has been inferred that the cerebellar cortex possesses relatively fewer inhibitory axodendritic synapses than the cerebral cortex (363). On the other hand, chloralose was shown to be a local activator of the cerebellar cortex and to determine a spread of its visual evoked potentials, in contrast to pentobarbital sodium (146, 147). Vestibular influences on cerebellar neurons were identified in toads (94), and convergences from different origins upon Purkinje cells were demonstrated in the frog (84). In the cat, responses evoked in crura I and II by stimulation of the contralateral pericruciate cortex were analyzed (400); for effective transmission of visual impulses from the colliculus to the cerebellum, chloralose was shown to facilitate a direct tectocerebellar route, instead of the normal

tectoreticulocerebellar pathway (148). Efferent projections have been traced from the fastigial nucleus to the labyrinth (87) and to the lateral reticular nucleus of the cat (428a). Influences from the anterior lobe on cells in Deiter's nucleus revealed a topographic organization (358). Flexor responses to stimulation of the rostromedial part of the nucleus interpositus in decerebrate cats also show a definite somatotopy (355) through the rubrospinal route. On the other hand, evidence of a complex action of the same nucleus upon red nucleus cells (*pars magnocellularis*) was obtained (10, 303).

Cellular mechanisms of cerebellar influences on motoneurons were investigated (411, see below). Further support was given to the concept of a functional opposition between the vermian and intermediate paravermian zones by studying anterior lobe influences on extensor alpha motoneurons in decerebrate cats (83), as well as the motor effects of combined cortical and cerebellar stimulations in free cats (317).

Other data deal with cerebellocortical projections in the cat. Paleo- as well as neocerebellar stimulation was confirmed to produce a generalized neocortical EEG arousal (259). Projections from the cerebellar auditory area into the cortical auditory or non-auditory fields partially involve the reticular formation (399). Cerebellar influences upon the hippocampus were also described (228), and projections from vermis (mainly paleocerebellar) and flocculonodular lobe traced to the limbic system proper (15) in the dog.

As to the complex problem of motor co-ordination, repetitive stimulation of the dentate nucleus in cats was shown to facilitate tremor induced by mid-brain tegmental stimulation (13). Motor disturbances and recovery after local ablations or nuclear lesions were described in dogs (181).

#### RHINENCEPHALON

Anatomical studies in the albino rat have described projections from the cingular area to several parts of the hippocampal system (436). Physiological methods led to identification of cranially as well as caudally directed connections through the dorsal fornix in the rabbit (105), and point-to-point connections between the two hippocampi through the ventral hippocampal commissure in the cat (16).

Because of the relative structural simplicity of the hippocampus, several investigations were concerned with basic processes underlying its activity, especially responses to stimulation of fornical or commissural afferents. Aspects thus studied concern the scarcity of inhibitory synapses in the hippocampus (361), the presence of strong posttetanic potentiation in response to repetitive afferent stimulation (86), a propagation into the apical dendritic tree of pyramidal neurons [postulated to be an abnormal process related to initiation of a convulsive activity (142)], and on patterns of responses of pyramidal neurons to stimulation of commissural afferents (17, 18). Enduring ("DC") hippocampal responses to local direct stimulation were also recorded and compared to similar phenomena in the neocortex (238).

The well-known particular feature of the hippocampal arousal pattern

in the rabbit hippocampus, i.e., appearance of rhythmic theta waves, may (415) or may not (233) also exist in the cat; in the rabbit, it is suppressed (as are the hippocampal sensory responses) by lesions in the septum or unspecific thalamic nuclei (140), and thus may depend upon a septal pacemaker (68).

Non-epileptogenic repetitive stimulation of the hippocampus was shown to increase for a limited time the amplitude of neocortical sensory responses in curarized unanesthetized rabbits as well as those with implanted electrodes (91); rhinencephalic structures, together with the pallidum, were also shown to act upon the midbrain reticular formation (2). Motor behavioral effects of local stimulation of the amygdala in the cat indicate a definite functional localization within this nuclear complex (422).

Rats' reactions to pain were not impaired by lesions placed in the limbic cortex, septal nuclei, or fornix (285, 286). In monkeys, however, at least one mechanism for emotional expression might be represented by a pathway from the septal nucleus to the hippocampus (427). In the central mechanisms for affective reactions, according to the review of Hunsperger (219), the rhinencephalic areas play an important role.

## EVENTS AND MECHANISMS AT THE NEOCORTEX

### BASIC PROCESSES

Data to be considered here could be located halfway between studies on basic neuronal processes not pertaining to the present review, and results more specifically concerned with interconnections or projections in the central nervous system. Bullock's (74) remarks "on old and new trends" in elementary neurophysiology, as well as reviews on central inhibitory processes by Beritoff (41) and by Fessard (153), provide interesting introductions to a study of central elementary mechanisms. Problems in interpretation and meaning of evoked potentials were also considered [Chang (291)], and Rosenblith (373) has discussed methodological problems related to their quantitative estimation.

Changes taking place in neuronal organization during postnatal development in the rat were described (133). Sholl (392) compared the "packing density" of neurons in visual and nonvisual areas in the cat. Precise data on axosomatic and axodendritic synaptic contacts were obtained by electron-microscopical studies of the rat's visual cortex (191). Physiological studies also deal with the problem of synaptic organization. Suggestions were again made that specific and nonspecific afferents have distinct modes of ending on cortical neurons (334), that individual fibers of the callosal system show a correspondence between depth of origin and depth of ending in the cortex, (183). The synaptic organization of the callosal link to corticospinal neurons has been studied (362). Through intracellular recording, new details were obtained on events in neurons from the somatosensory cortical area and effects of strychnine (276). Simultaneous recording with multiple microelec-

trodes from neighboring loci in the cortex, and also in the thalamus, led to the postulation of a continuous circulation of neuronal activity within closed loops (425). Cortical responses to direct electrical stimulation have also brought valuable information in this field: identification of their successive components, possible role of apical dendrites, and regional pattern differences to be correlated with histological characteristics of the active substrate (340, 381, 402).

On the other hand, the properties and significance of long-lasting ("DC") shifts were considered. From the effect of transcortical polarization upon cortical evoked potentials, Caspers (89) concluded that "dendritic" components (negative phase) represent a modulation in amplitude of the DC potentials. Other experimental data on the optic cortex in the rabbit and cat (177, 178, 348) alternatively suggest that negative long-lasting aftereffects and negative dendritic spikes are distinct postsynaptic events.

On the basis of data from topical actions of drugs such as  $\gamma$ -aminobutyric acid, strychnine, and curare, together with those from deep laminar recording, Purpura *et al.* (352) further developed their tentative identification of the elements in synaptic central organization, with differentiation of excitatory and inhibitory components (364, 387, 396). A number of other pharmacological analyses led to the conclusion that the central (facilitatory or depressant) effects of various injected drugs are, in fact, the result of combined multivalent actions; such data report on curare (341),  $\gamma$ -aminobutyric acid (407, 443), 5-hydroxytyramine (251), cortisone (109), and lysergic acid diethylamide (48, 252).

It is known that surgical isolation of cortical tissue increases its sensitivity to various actions. That this occurs even after partial deafferentation, with a maximum after two to four months, was shown (158). Isolation rather than ischemia or injury should be the determinant factor for hypersensitivity, according to Echlin (139). Inhibitory effects of "I factor" and  $\gamma$ -aminobutyric acid were compared on isolated slabs (108) and, on the other hand, the action of anticholinesterase indicated that a cholinergic mechanism might exist at the cortical level (124).

Marshall (297) has reviewed cortical spreading depression, "a deceptive phenomenon which has confused experiments on physiology of cerebral cortex for years." Recent studies dealt with activation of depression foci in the rat (76), and the parallel time courses of spreading depression and potassium outflux from the rabbit cortex (62).

Epileptogenic cortical foci resulting from different topical treatments may also represent a useful approach to basic mechanisms. Cortical spread of penicillin-convulsive activity was shown to take place via the subcortex (260); anticonvulsant drugs were tested in rabbits and cats on chronic lesions caused by ethylchloride (318, 321). A microelectrode investigation revealed the progressive changes in cortical unit discharges which develop as an electrocortical seizure develops (169). Regional differences in neocortical

susceptibility to "after-discharge" elicited by direct stimulation were observed in the cat, with a maximum for the middle sylvian and suprasylvian gyri (164). Morrell's (319) interesting and puzzling attempts to individualize "lasting changes" in synaptic cortical processes as a model of elementary learning by using chronic epileptogenic lesions should also be mentioned here.

#### NONPRIMARY PROJECTIONS AND ASSOCIATIVE SYSTEMS

Sensory projections toward nonprimary projective fields were identified in several cortical regions and under different conditions.

Additional evidence has appeared suggesting that visual as well as acoustic impulses impinge upon the motor cortex; an acoustic stimulation is capable of modifying the excitability of the motor cortex in the unanaesthetized cat (347). An acoustic focus in the cat motor cortex was revealed by macro-electrode recording (413); further, microelectrode exploration encountered in this area a majority of "polysensory units", responding to somesthetic as well as to visual and acoustic stimuli (80).

For "associative" sensory projections various foci were also mapped out: somesthesia projects to the prefrontal cortex of the monkey (12) and vision and somesthesia on the anterior part of the mesial cortex in the cat (218); suprasylvian foci for audition, independent of primary projection fields, exist in the cat (413), as do suprasylvian responses to light and sound in dogs with implanted electrodes (176).

In addition, primary projective fields may also be subject in certain conditions to a "cross-modality" activation. Visual neurons were thus shown to respond in the cat to labyrinthine excitation (194 to 196) and in the rabbit to acoustic, olfactory, and painful stimulation (280).

#### INTERCORTICAL RELATIONSHIPS

Callosal volleys act upon cells of origin of the pyramidal tract of the cat producing either long-lasting inhibition (28) or activation, or both, through a complex many-wayed synaptic system (362). Stimulation of one cortical area evokes a complex response in the symmetrical area, early components of which arise from the corpus callosum, and late ones from mediation through a subcortical "diffuse multisynaptic structure" (377). Transmission of after-discharges induced in one temporal lobe to the opposite one was shown to occur in the monkey mainly by way of the anterior commissure; other commissural systems did not play a significant role (353). In the same animal, complex corticocortical associative connections have been traced anatomically from the projection field of the medial geniculate body, to area 22 and from there to other cortical zones (frontal, parietal, etc. (288)).

Electrophysiological studies in the cat gave information on connections from somatic II to ipsilateral somatic I area (331), from posterior to anterior sigmoid gyrus (330), and between auditory I and II projection fields (130).



## ELECTROCORTICAL RHYTHMS

Tunturi (419) presented statistical data based on a spatial analysis of the spontaneous electrical activity from the middle ectosylvian gyrus in barbitalized dogs. Ontogenesis of cortical spontaneous and evoked activity has been studied in fetal sheep (42) and in a newborn kitten (298, 299).

In man, spatiotemporal distribution of alpha-rhythms was considered (103) and occipital rhythms classified (6); rolandic "wicket rhythms" were shown to be blocked by passive or reflex movements as well as by spontaneous voluntary movements and also by tactile stimuli and mental activity (96). According to Larsson (273), "nonspecific" EEG responses to strong and unexpected stimuli, recorded from the human scalp, cannot be an artefact caused by "startle" movement, as is sometimes suggested; their magnitude appears moreover to be related to the degree of significance of the stimulus (272). Correlation analysis (32, 33) was applied to the study of electrocortical frequency-specific responses during conditioning in rabbit and cat (320).

## MECHANISMS AND STRUCTURES FOR GENERAL CONTROL

## ASCENDING RETICULAR FORMATION

Reviews were published and symposia held in the U.S.S.R. on functions of the reticular formation (22, 403).

*Reticular neurons.*—Considering reticular responses to sensory inputs, Schlag *et al.* (383) showed that the more a reticular unit is firing spontaneously, the less it appears responsive to peripheral stimuli; and Feldman *et al.* (150, 151) demonstrated that under increasing barbiturate anaesthesia, gross responses to sciatic stimulation first decrease and disappear, then are replaced by a long-latency wave, possibly related to the secondary discharge of Forbes. Data and hypotheses on the differential action of drugs such as reserpine or chlorpromazine were reviewed and discussed (165, 246, 261); that chlorpromazine acts by enhancing the reticular "filtering" of sensory inflow has been suggested (248). Various observations again emphasized the particular sensitivity of reticular neurons to various drugs, especially  $\gamma$ -aminobutyric acid (247, 406 to 408, 417; see also 141). Even minute amounts of barbiturates or convulsants were shown to be effective when injected directly into the hypothalamus (168).

*Extrinsic influences upon reticular neurons.*—Subthalamic structures exert a tonic excitatory influence on the midbrain reticular formation, as Adey & Lindsley (3) showed with lesion experiments in cats and monkeys. The amygdala and pallidum also act on reticular neuron firing patterns, and the pallidum facilitates tremor induced by reticular stimulation; these results are found in animals (2, 14) and also in man (4), thus posing the interesting question of the possible mechanism of tremor at rest as a result of interplay of rhinencephalic and pallidal actions.

The neocortex influences reticular neurons (or "diffuse" thalamic structures): arousing stimuli, acting through the reticular formation, become less effective during cortical spreading depression in the rat (434); responses are affected by cortical stimulation in the cat (333), and spindling is absent in the thalamus of decorticated rabbits (386).

*General ascending reticular influences; non-specific thalamic nuclei.*—Some recent data related to this topic were discussed by Magoun (292). Jasper (in 291) outlined established facts on the organization of the "diffuse" thalamic system, a group of structures which are usually considered at least one possible route for ascending non-specific influences. Among recently discovered facts the orienting reaction in the rabbit was shown to be linked with reticular and cortical synchronization of spontaneous rhythms (339). Intralaminar thalamic stimulation in the unanaesthetized monkey could synchronize activities from two independent cortical epileptogenic foci (311). Arguments, chiefly based upon differential effects of drugs (chlorpromazine, reserpine, imipramine), were developed for considering the mesencephalic activating and diencephalic recruiting mechanisms as antagonistic systems (263, 315, 316, 414). Reticular effects on sensory channels will be considered below.

#### SUPRASPINAL CONTROL OF REFLEX ACTIVITY

Stepping movements were observed after decerebration in guinea pigs, and facilitatory or inhibitory effects from stimulation of remaining reticular structures considered (107). According to Butkhuvi & Narikashvili (82), the reticular mesencephalic facilitatory and bulbar inhibitory actions upon reflexes resist narcotic drugs differently; reflex facilitation thus dominates in unanaesthetized animals, whereas a generalized inhibitory effect emerges as narcosis develops. Reticular facilitation or inhibition (423) was found to modify, in one or the other sense, the excitability of motoneurons as tested by evaluating the threshold for direct intracellular stimulation. However, no perceptible variations in resting potentials or amplitude of intracellular postsynaptic potentials were noticed in the same conditions (254); other processes might thus account for facilitation (such as earlier rise of the action potential from postsynaptic potential) and also for inhibition.

Another point of interest, in part distinct from the preceding approach, concerns the observation of a striking difference between decerebrate and spinal cats (138). It has been shown (281) that Group II and III muscle afferents, skin, and high-threshold joint afferents are considerably more efficient in producing flexion reflexes after elimination of the brainstem. A tonic inhibition of the interneurons mediating the general flexion reflex might thus exist in decerebrate preparations [and is also reproduced in the spinal cat by conditioning with a volley from a cutaneous nerve (265)]. A similar effect was found for interneurons of the Ib excitatory and inhibitory pathway. Other results show that the pathway for spinal inhibition of interneurons involved in both cases (Ib and flexion reflexes) passes through the

dorsal half of the lateral funiculus, that inhibition is not affected by the cerebellum or vestibular nuclei, and that two distinct pathways may mediate inhibition to extensors and excitation to flexor motoneurons (209). Other descending actions were analyzed. Studies by Gernandt *et al.* (171, 172) concern descending vestibular influences acting on spinal motoneurons; stimulating vestibular nerve may either evoke a motor outflux or condition segmental spinal reflexes. The relative parts played by vestibulospinal or reticulo- and propriospinal pathways in transmitting these influences were also considered (170). Cerebellar control was studied in the decerebrate cat: flexor alpha-motoneurons are inhibited by stimulation of all subdivisions of the vermal cerebellum, whereas alpha extensor cells are potentiated from the vermal strip and inhibited from the paravermal (83). Intracellular recordings from extensor motoneurons further led to the correlation of cerebellar inhibition (or facilitation and rebound effects) with increase (or decrease) of their membrane potentials (411). In unanaesthetized monkeys, the precentral cortex acts on lumbosacral motoneurons by both facilitatory and inhibitory actions which are identical for both flexors and extensors, reciprocal innervation thus appearing principally as a property of segmental organization (360).

#### UNSPECIFIC CONTROL OF SENSORY CHANNELS

Two distinct groups of observations belong to this heading: control (especially reticular) may be exerted at the receptor level or at the first relay and so be "centrifugal", or at higher stations, thalamic or cortical, and so represent an "ascending" influence.

Results and ideas on centrifugal control of afferents were reviewed and discussed [Livingston (291); Hagbarth (200)]. The anterior cerebellum and sensorimotor cortex were shown to be stations for a centrifugal modulation of spontaneous unitary firing in ascending spinal tracts (202). The pyramidal tract may also (290) act upon dorsal column nuclei through a direct pathway (not including the reticular formation), which has been traced anatomically (266). Transmission in three spinal ascending pathways influenced by afferents giving rise to the flexion reflex was shown to be tonically inhibited from supraspinal centers (211). Variations in the amplitude of sensory peripheral responses accompanying attention, habituation, or arousal were again observed in normal animals and their mechanism discussed (173). Variations in olfactory lobe (206) and retinal (346) responses thus observed under chronic conditions were considered to depend upon a brainstem reticular action. Other data tend to minimize the importance of reticular centrifugal control. Visual habituation was recently shown (90, 293, 294) to take place at first at the cortical level before reaching the subcortex and, on the other hand, to exist in midpontine and even "*cerveau isolé*" cats, the reticular activating system thus seeming not to be necessary for maintenance of sensory habituation. These facts were also clearly analyzed and discussed by Moruzzi (322). Other data (216) also indicate that the disappearance of

cochlear nucleus responses to clicks during arousal could, in fact, result from contraction of the middle ear muscles, and not from a reticular inhibitory influence.

Now, considering ascending influences, it is well established that the reticular formation exerts a depressive action upon thalamic and cortical responses to stimulation of receptors. That this was also the case for potentials caused by stimulation of the cat tongue has been shown (23). Besides, it was already known and has recently been confirmed [(335); see also Magoun (292)] that responses to stimulation of sensory nerves or central tracts are, in opposition to "natural" responses, facilitated by reticular activation. As a tentative interpretation of this difference, Bremer & Stoupe (60) ascribe "paradoxical" inhibition of "natural" responses to a process of occlusion masking reticular facilitation. The latter phenomenon is abolished by administration of barbiturates, but not by chloralose nor by atropine (59). To Long (281), conditioning of somatic or visual potentials by the reticular formation appeared either facilitatory or inhibitory, depending on frequency of stimulation. After observing fluctuations of evoked responses to light at different stations of the optic pathway, Naquet *et al.* (332) assumed that reticular control might be first exerted upon higher levels, mainly cortical [see also (24) and (81)].

#### SLEEP AND WAKEFULNESS

"*Encéphale isolé*" animals usually considered as predominantly awake may, on the contrary, develop persistent sleep spindles in long-term records, according to Wang *et al.* (431). On the other hand, Jouvet *et al.* (234) confirmed that, during natural sleep, "paradoxical stages" with high-frequency, low-voltage rhythms may occur in the cat.

As previously reported by Moruzzi's group, cats with a complete mid-pontine transection ("midpontine pretrigeminal" preparation) show persistent low-voltage fast EEG rhythms as well as ocular behavior suggestive of wakefulness. However, recent data indicate that a midpontine preparation may present EEG sleep patterns under particular conditions, such as acute ischemic deafferentation of the retina [presumably suppressing a tonic afferent discharge (25)], keeping the animal for a certain length of time under steady illumination (27), or submitting it to flickering light (295). The previously postulated existence of a synchronizing center in the lower brainstem was submitted to further analysis by Cordeau & Mancia (104) who performed chronic midpontine pretrigeminal hemisections and could thus induce an asymmetry in the degree of electrocortical synchronization of both hemispheres. As an additional indication, low-frequency stimulation of the inferior reticular formation produced a slowing and an increase in amplitude of cortical rhythms (149).

From a comparative study on normal, decorticated, or mesencephalic cats with implanted electrodes, two physiological sleep mechanisms were postulated by Jouvet *et al.*, one in the lower brainstem (232, 234), the other

telencephalic and implicating a descending inhibitory influence from the cortex upon the reticular system. It was finally suggested (233) that sleep inhibition may also originate from the rhinencephalon. Rabbits and birds under hypnosis showed similarities to natural sleep in EEG patterns; EEG activation by intercurrent stimuli with or without behavioral recovery, and habituation of arousal were also observed (394).

#### NEW TECHNIQUES

Many efforts were directed toward designing or improving methods for the instrumental analysis of electrical nervous activity, such as analyzing frequency of spontaneous rhythms, averaging evoked potentials, detecting small amplitude responses embedded in background noise, etc., and discussing the value and perspectives of available techniques (32, 75, 258, 310, 373).

A volume by Donaldson (128) provides a most useful repertory of electronic devices for biological research, including neurophysiological techniques. Among the new apparatus proposed are transistor stimulators (31, 121), a microelectrode manipulator (175), a multiple electrode holder for cortical or subcortical recording on the rabbit (250), and a device for a single-unit recording in the subcortex of the unrestrained cat (214).

Refinements of the stereotaxic technique should also be mentioned, such as reaching the hypothalamus in the dog (20), checking alignment of electrodes (442), controlling planes of references in the monkey (388), and making deep lesions (174). Two stereotaxic atlases of the dog's brain have appeared (5, 277). Precise directions for stereotaxic approaches in man are also available (30, 122, 382), together with documentation for descriptive anatomy of the human brain.

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# VISCERAL FUNCTIONS OF THE NERVOUS SYSTEM<sup>1</sup>

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A few words of retrospect are usually allowed as an introduction to these laconic presentations. In the reviewer's opinion, the problem of adequate communication is one of the most pressing in today's scientific medicine. The utmost should be done to give the physiologist both the useful facts of the literature and the time to think and experiment. This requires an organized bibliographical service more effective than those usually available, including continuous collection and indexing of all useful reports from the 5000 medical periodicals, as a complement to the current reviewing activities. Authors' own abstracts should be prepared and codified to suit an automatized indexing system. At last, a uniform system of abbreviations and symbols should be accepted to unburden the investigators of the present variability of these formal details. These problems are present not only in physiology; their solution should be worthy of a united effort of nations.

*Reviews.*—The year's literature contains many valuable reviews within the present field. In the first place, the American Physiological Society's new *Handbook of Physiology* should be mentioned. The first two volumes of the Neurophysiology Section contain articles on autonomic neuroeffector transmission [von Euler (1)], thermal sensations [Zotterman (2)], central control of receptors and sensory transmission systems [Livingston (3)], central autonomic mechanisms [Ingram (4)], peripheral autonomic mechanisms [Hillarp (5)], central control of pituitary secretion [Harris (6)], neurosecretion [Ortmann (7)], the neural control of respiration [Oberholzer & Tofani (8)], central cardiovascular control [Uvnäs (9)], central control of digestive function [Eliasson (10)], central regulation of body temperature [Ström (11)], regulation of feeding and drinking [Brobeck (12)], central control of the bladder [Ruch (13)], the reticular formation [French (14)], the intrinsic systems of the forebrain [Pribram (15)], the cingulate, posterior orbital, anterior insular, and temporal pole cortex [Kaada (16)], the hippocampus [Green (17)], and the amygdala [Gloor (18)]. Other reviews cover the vasomotor regulation of cutaneous circulation [Hertzman (19)], the control of peripheral resistance in major systemic vascular beds [Green & Kepchar (20)], gastric emptying and secretion in man [Hunt (21)], the hemodynamics of hypertension [Freis (22)], and a bibliography on the effects of cold on man [Culver (23)]. Further reviews describe the nervous mechanism of taste [Zotterman (24)], the structure of afferent and efferent innervation of the cardiovascular system [Ortmann (25)], neuroeffector transmitters [Holtz (26)], central cardiovascular control [Oberholzer (27)], circulatory reflexes from the arterial receptive

<sup>1</sup> The survey of literature pertaining to this review was concluded June 1, 1960.

mechanisms [Neil (28)], and the cardiac and venous receptive mechanisms [Kramer (29)], the efferent nervous control of the cardiovascular system [Folkow (30)], dysfunctions of cardiovascular nervous control [Mechelke (31) and humoral control [Schwiegk (32)].

Among the fields beautifully covered by experimental reports, the problems of neurotransmitter formation, localization, and action, of hormonal conditioning of nervous action, of nervous and humoral control of myocardial function, and of temperature regulation have attracted the special interest of the reviewer, particularly when related to human physiology and pathophysiology.

#### CEREBRAL SYSTEM

The relationship between certain clinical and psychological variables on the one hand and signs of visceral activity on the other has been further investigated. Dykman *et al.* (33) found that electrical skin resistance of male students was better than heart rate and respiration as an index of sympathetic autonomic activity, activated by tone or emotional questions. Such psychological attitudes or variables as apprehension or intellectual proficiency were correlated with a high activity level and consistency of responses, while the degree of anxiety was not. Chlorpromazine lowers and methamphetamine tends to heighten the activity level of electrical skin resistance [Dureman (34)]. Tension-restlessness in psychiatric patients and the anxiety syndrome were correlated with the increase of volume pulse in finger plethysmography caused by sodium amytal injection [Nilsson (35)]. Psychiatric patients with mania showed a higher than normal rate of urinary excretion of epinephrine, both under basal conditions and during insulin hypoglycemia; patients with endogenous depression as well as those with senile dementia showed subnormal values while schizophrenics did not deviate from normal [Bergsman (36)].

Cerebral localization of visceral control has attracted continued interest. Electrical stimulation of the amygdala, the stria terminalis, and rostral hypothalamus in unanesthetized cats evoked affective reactions characterized first by growling, then by shrieking and flight [Fernandez de Molina & Hunsperger (37)]. Behavioral attention, fear, anger, and various somatomotor and autonomic effects were elicited from the amygdala with signs of functional localization, in experiments performed mostly in unanesthetized cats [Ursin & Kaada (38)]. Cats with chronic lateral hypothalamic electrodes implanted could learn both to carry out self-stimulation and to avoid electrical stimulation through the electrodes, which produced a rage response [Brown & Cohen (39)].

A new procedure for chronic implantation of electrodes into the hypothalamus through the diasphenoid bone in dogs was described [Andersson (41)]. The spontaneous electrical activity of the ventromedial hypothalamic nucleus in unanesthetized cats showed a pattern during sleep similar to that of other regions, but during arousal a characteristic 20 to 35 c.p.s., 40  $\mu$ v pat-

tern appeared [Brooks (42)]. Sciatic nerve stimulation in the cat produced a short-latency evoked potential in the lateral posterior hypothalamus and a long-latency potential in the medial anterior hypothalamus [Feldman *et al.* (43)]. Cortical direct pathways to the hypothalamus in the rabbit were ipsilateral; they emerged principally from the paleocortex, none coming from the neocortex, and passed via the medial forebrain bundle or the fornix to terminations mainly in the posterior region or mamillary body [Lundberg (44)].

The local administration of ammonium chloride (independent of  $p\text{CO}_2$  or pH) into the fourth ventricle in cats, which were without functioning peripheral chemo- and baroreceptors as a result of the administration of chloralose-urethane, produced an increase in respiratory tidal volume and in arterial blood pressure, which was followed by a depression when higher doses were given [Loeschke & Katsaros (47)].

#### CIRCULATORY SYSTEM

*Receptors and afferent systems.*—Degenerative changes of the carotid sinus baroreceptive region were found in hypertensive patients [Hilgenberg (48)]. Aortic and carotid baroreceptors were afferently active in newborn rabbits, producing a moderate reflex depressor effect although their arterial pressure is regulated at a low level [Downing (49)]. Local arterial pressure decrease, as well as denervation of the thyrocarotid arterial junction, produced circulatory effects in dogs, suggesting the presence of baroreceptors at this junction [Gann & Bartter (50)]. Baroreceptors were demonstrated, by afferent recording, in the pulmonary artery of dogs at its bifurcation; most of them showed a systolic discharge [Coleridge & Kidd (51)].

Reflex peripheral vasodilatation and moderate bradycardia were evoked in dogs by a sudden increase of left ventricular systolic pressure, which was produced by aortic root compression, a constant pressure level being maintained at peripheral baroreceptive sites and in the left atrium. This suggests that baroreceptors are present in the wall of the left ventricle [Douthell & Kramer (52)]; afferent signalling took place via the vagus nerve. Similar reflex effects were produced by local increase of the pressure level in the left atrium and the pulmonary venous-capillary system. Evidence for the existence of baroreceptors in the dog's left ventricular wall, producing reflex bradycardia and vasodilatation mediated via the vagus, was also obtained by Aviado & Schmidt (53). Afferent stimulation of the vagosympathetic trunk in the neck of dogs produced an arterial pressure increase which was not changed after unilateral chronic cervical-thoracic sympathectomy; this indicates that the reflex response was mediated via the vagus [Kelly (54)]. In the cat's vagal root, the afferent fibers in the rostral filaments were activated mainly in cardiac rhythm, while those of the caudal filaments discharged exclusively in the respiratory rhythm [von Baumgarten *et al.* (55)].

Afferent impulses from the kidney of the cat were recorded from the nervous plexus around the renal artery [Pines (56)]. A few fibres discharged in

the cardiac rhythm and it was suggested that they acted as vascular mechanoreceptors.

*Central control.*—Controlled emotional arousal evoking anxiety or anger in male students, resting supine, produced circulatory reactions related to the intensity but not the quality of the emotion; under strong emotion, heart rate (+28%), stroke volume (+26%), and arterial pressure (+11%) increased moderately, cardiac output (+63%) markedly [Bogdonoff *et al.* (57)]. Electrical stimulation of the posterior orbital gyrus in the cat had little effect on arterial pressure unless a pressure fall was first initiated by carotid blood heating; then a rise of pressure to the initial level was produced [Newman & Wolstencroft (58)]. The after-discharges following electrically induced seizures from the anterior and central regions of the amygdala in unanesthetized cats were accompanied by a fall of arterial pressure, but no change of heart rate or rhythm occurred [Andy *et al.* (59)].

Stimulation of regions around the sigmoid gyrus in the cat sometimes produced ECG changes, principally T-wave depression or inversion, even without accompanying changes of arterial pressure [Kenedi & Csanda (60)]. Stimulation of the lateral and posterior hypothalamus in cats was even more effective in producing ECG alterations, such as changes in amplitude of QRS and T, or ectopic impulse formation, usually with tachycardia [Fuster & Weinberg (61)]. This result has a bearing on the problem of central control of myocardial excitability and force of contraction (see below).

Just caudal and ventral to the anterior commissure in cats, a restricted region was found from which drastic inhibition of sympathetic vasoconstrictor and cardioaccelerator tone could be elicited [Folkow *et al.* (62)]. In chloralose-treated cats, the bradycardia evoked by stimulation of the dorsal vagal nucleus and the arterial pressure increase elicited from medullary vasomotor areas were greatly diminished by *d*-tubocurarine (e.g., 0.1 mg./kg. intravenously) by a direct action, while similar cardiovascular responses from the hypothalamus were only slightly influenced [Peiss & Manning (63)]. The ability of spatial and temporal summation of afferent signals from various cutaneous nerves, when combined with hypothalamic stimulation, to evoke sympathetic effector reactions in the nictitating membrane, the arterial pressure, and the heart rate was demonstrated by Gellhorn (64). Activation of a single effector system sometimes resulted, demonstrating that partial activation may occur. Hypothalamic stimulation in the rat usually raised the arterial pressure; however, changes in the amplitude or frequency of stimulation or in the basal vasoconstrictor tone sometimes led to a fall in pressure [Scherrer (65)].

By means of ischemic decerebration and serial transections of the brainstem in vagotomized cats, evidence was found for the existence in the brainstem of a cardiovascular depressor region rostral to the midpontile level and an augmentor region caudal to that level; these regions influenced heart rate and arterial pressure [Glasser (66)]. Stimulation of ventrolateral regions of the medulla in vagotomized cats under pentobarbital, and of dorsal medul-

lary regions under chloralose, produced tachycardia after a short latency (1 to 5 sec.), but the cardiac response was eliminated after electrolytic lesions or transections at the midcollicular level, indicating that the impulse stream was relayed to supramesencephalic regions [Peiss (67)]. Bilateral vagotomy or local destruction of the vagal nuclei in guinea pigs was followed within eight hours by lethal pulmonary edema; this result was also obtained in decerebrate guinea pigs [Borison & Kovacs (68)].

*Ganglia and efferent systems.*—The existence of an efferent preganglionic sympathetic system with inhibitory effect on ganglionic synaptic transmission was suggested as an explanation of changes of preganglionic and postganglionic activity after pseudorabies infection in rats [Dempsher *et al.* (70)]. The cholinesterase activity of the cat's perfused superior cervical ganglion was abolished by tetraethylpyrophosphate in a concentration ( $10^{-4}$ ) which did not influence the response of the nictitating membrane to supramaximal preganglionic stimulation and only moderately potentiated the response to submaximal stimulation, while the effect of a single dose of acetylcholine added to the perfusate was potentiated a hundred-fold [Fehér & Bokri (71)]; continued administration of acetylcholine in a moderate dose desensitized the ganglion to this substance but ganglionic transmission of preganglionic impulses remained unaltered [Fehér & Bokri (72)]. The blocking effects of atropine and decamethonium on postganglionic activation were different for acetylcholine and for preganglionic stimulation. As an explanation for these results Fehér & Bokri suggested that the ganglion may possess two types of acetylcholine "receptor" systems.

High cervical stimulation of the vagus in cats did not produce any significant chronotropic or inotropic effects on the atropinized, isolated, and perfused heart, but stimulation of the thoracic vagus caused significant cardiac stimulation [Benítez *et al.* (73)]; the latter effect was not influenced by high vagotomy but was abolished by cervical and upper thoracic sympathectomy, demonstrating that cardiac sympathetic fibres join the vagal trunk at its entrance into the thorax. Similar experiments on the isolated rat heart did not demonstrate any sympathetic fibres in the vagal trunk [Benforado (74)].

Reflex vasoconstriction in the lower limbs was produced by cooling of one upper limb in normal individuals but was absent after sympathectomy or high spinal transection in paraplegic patients [de Crinis *et al.* (75)], confirming that these reactions are mediated by the sympathetic outflow. Winbury (76) found that 1,1-dimethyl-4-phenylpiperazinium had a direct vasodilator effect demonstrable in the chronically denervated hindlimb of the dog, resistant to pharmacological blocking. In the innervated limb, the effect was vasodilator and was blocked by cocaine but not by atropine or pretreatment with reserpine; after botulinus toxin or hexamethonium treatment, the dilator effect was converted to constrictor, and this vasoconstriction blocked by cocaine or phentolamine. Therefore, Winbury suggested that the vasodilator effect in the innervated limb was indirect and, like the effect of nicotine, acting on a separate nerve pathway, neither cholinergic or adrenergic, while



the vasoconstrictor effect might well be explained by transmitter release from vasoconstrictor nerve endings. A similar problem was studied by Hilton (77) in which the cat's femoral artery was found to dilate in response to muscular contractions in the ipsilateral hindleg, this effect being mediated by the smooth muscle cells of the media (see also p. 312). A local impulse conducting system (nervous or muscular) in the arterial wall was also suggested by observations on the transmission of pressure waves along human arteries [Heyman (78)].

A neurohistological study of the terminal structure of the autonomic innervation in intestines, uterus, and iris in rats, rabbits, and cats suggested the presence of an "autonomic groundplexus" [Hillarp (79)].

*Transmitter action.*—The action of acetylcholine, especially on the heart, has been further investigated. Spontaneous release of acetylcholine in the dog's right atrium, both during arrest and while beating, was demonstrated by its effects on the electrical activity of single fibres, i.e., shortening of the action potential, increase of the resting potential, and suppression of the slow diastolic depolarization in sinus fibres; these effects were counteracted by atropine and increased by cholinesterase inhibitors [Trautwein *et al.* (80)]. The shortening of the atrial action potential in the rat's heart during vagal stimulation or acetylcholine administration took place independent of the rate of contraction when the atrium was driven by electrical stimulation [Biersteker *et al.* (81)]. Acetylcholine depressed or blocked transmission from atrium to ventricle in the rabbit heart by action on atrial fibres at the margin of the atrioventricular node; these fibres developed slowly rising, low, and fragmented action potentials with acetylcholine [Cranefield *et al.* (82)].

Acetylcholine increased both influx and efflux of  $K^+$  in isolated rabbit atria, the influx rising more rapidly than the efflux at surrounding  $K^+$  concentrations higher than the flux equilibrium value of about 4.0 to 4.5 mM; flux equilibrium without acetylcholine occurred at 6.0 to 6.5 mM [Holland *et al.* (83)]. Rayner & Weatherall (84) reported that acetylcholine in rising concentration accelerated first the efflux and then also the influx of  $K^+$  in rabbit atria and that an increased net loss from the tissue occurred at an outer  $K^+$  concentration of 5.8 mM. Acetylcholine-induced fibrillation in rabbit atria was terminated by administration of deoxycorticosterone and quinidine, which were also found to depress  $K^+$  efflux and lengthen the functional refractory period [Briggs & Holland (85)]. Changes of  $Na^+$  and  $Cl^-$  fluxes were also observed in atrial fibrillation caused by acetylcholine [Briggs & Holland (86)].

The action of the catecholamines has been further elucidated, as discussed in the chapter on Peripheral Circulation (p. 295). Additional studies of interest are reported here. A close analysis of the temporal development of epinephrine action demonstrated differences from the vasodilator action of ATP; a blood lactate increase up to 5 to 6 mM did not influence blood flow. Norepinephrine first inhibited the circulatory convection of intradermal  $Na^{24}$  in the dog's hindleg at an intravenous infusion rate of 0.3  $\mu g.$  per kg.

min. which also began to increase arterial blood pressure; in response to epinephrine, the skin blood flow index was decreased by smaller doses (0.05  $\mu$ g. per kg. min.) [Widmer *et al.* (95)]. The vasoconstrictor effect on the human forearm of continued infusion of epinephrine was antagonized by chlorpromazine and phenoxybenzamine, revealing a sustained vasodilator effect [de la Lande & Whelan (96)]. The vasoconstrictor effect of norepinephrine was lessened by arterial infusion in the dog's forelimb, in which it caused arteriolar vasodilatation by a direct action [Emanuel *et al.* (106)].

The contracting effect of norepinephrine on muscle strips from the rat's aorta was increased by alkalization and decreased by acidification of the medium [Tobian *et al.* (107)], while its effect on strips from the dog's aorta increased with the surrounding sodium ion concentration [Yamabayashi & Hamilton (108)]. The arterial pressure rise and increase of blood flow in the cat's hind quarters, caused by norepinephrine infusion, were depressed by a reduction of artificial respiration [Dunér & von Euler (109)]. The effect of norepinephrine on the intact dog's arterial pressure and on the heart-lung preparation's cardiac output was not influenced by hydrocortisone or aldosterone administration, even after adrenalectomy [Small *et al.* (110)].

Norepinephrine increased pulmonary arterial pressure in the dog only after spinal section and vagotomy; 5-hydroxytryptamine had this effect also in the innervated dog [Nahas & MacDonald (111)]. The effect of epinephrine and norepinephrine on the pulmonary circulation in the innervated dog, that is, a decrease of flow and pressure, was attributable to reflex bradycardia and peripheral vasodilatation evoked from the baroreceptors by increased carotid arterial pressure [de Burgh Daly & Luck (112)]. Infusion of these substances decreased jugular vein outflow in the intact dog, but as lateral sinus outflow from the intracranial structures did not change, the former effect was attributed to extracranial vasoconstriction [McClure & Green (113)]. The implications of this result were judged to be of importance for evaluation of measurements of cranial blood flow in human subjects. Intravenously administered epinephrine and norepinephrine did not change cranial blood flow or oxygen uptake [Fazekas *et al.* (114)], even during the increase in arterial pressure.

Norepinephrine infusion prolonged survival time in dogs in oligemic shock [Fozzard & Gilmore (115)]. Isoproterenol produced a shift of blood from the forearm via venous constriction, and a fall in peripheral venous and effective atrial pressures [Eckstein & Hamilton (116)].

In the human brain, norepinephrine was present mainly in the brainstem, dopamine mainly in the corpus striatum [Bertler & Rosengren (117)]. Chronic cervical sympathectomy did not influence the catecholamine content of the rat's brain [Bertler & Rosengren (118)]. Epinephrine, norepinephrine, and hydroxytyramine were present in the rabbit's brainstem in the proportions of 1:4:5, evenly distributed between the cytoplasm and a mitochondrial cell fraction [Weil-Malherbe & Bone (119)]. After reserpine administration the three amines disappeared at similar rates, more quickly

from the cytoplasm than from the mitochondrial fraction; thereafter, the injection of dihydroxyphenylalanine caused a considerable elevation of hydroxytyramine concentration above the initial ("control") level before administration of reserpine, while the concentrations of epinephrine and norepinephrine returned to the control level within one hour, with similar distribution between the two cellular fractions. Also, without reserpine treatment, the injection of dihydroxyphenylalanine increased hydroxytyramine concentration above control level while epinephrine and norepinephrine concentrations were unaffected.

Intravenous infusion of phenoxybenzamine abolished the inhibitory effect upon the patellar reflex exerted by the bulbar reticular formation in the cat, while subsequent infusion of epinephrine restored the effect; local administration of epinephrine suggested the existence of epinephrine-sensitive regions in the medulla [Cranmer *et al.* (120)]. Intravenous epinephrine also produced an augmentation of the patellar reflex of the cat by a direct or spinal action, as judged on the basis of spinal transection experiments [Ten Cate *et al.* (121)].

Intravenous epinephrine produced a decrease of the plasma volume in the hen, while the red cell volume remained constant, the effect being apparent as an increase of peripheral hematocrit to a maximum within 20 to 30 min. and a return within 1 to 2 hr. [Tapper & Kare (122)]. The epinephrine antagonist phenoxybenzamine caused an increase of the plasma volume in the chicken, while pentolinium and tetraethylammonium had no such effect [Williams & Rodbard (123)].

The increased water content of the skin after thermal injury in the rat was prevented by amine oxidase inhibitors, but not after pretreatment with dibenamine, suggesting that after thermal injury an increased local destruction of epinephrine participates in the edema formation [Spector & Wiloughby (124)]. Norepinephrine produced a conspicuous increase in renal vascular resistance in the dog, with a decreased blood flow and increased average transit time, and with a small increase of renal blood volume, suggesting that vasoconstriction takes place at least partly in resistance vessels distal to glomerular or peritubular capillaries with a capacitive function [Mehrizi & Hamilton (125)].

The 24-hour urinary excretion of labeled substances after infusion of labeled norepinephrine in human subjects included norepinephrine (4%), normetepinephrine (3% free, 10% conjugated), and 3-methoxy-4-hydroxymandelic acid (32%), 67% of the infused radioactivity being recovered [Goodall *et al.* (128)].

*Nervous control of the heart.*—Heart rate was variably influenced by a rise of atrial pressure caused by intravenous infusion after vagotomy, atropine, or ganglion blockade, in the dog with the chest either open or closed. A "Bainbridge reflex" was therefore not present, and it was suggested that the influence of atrial distention on heart rate depends on a direct mechanical action on the pacemaker [Pathak (129)]. Systemic hypoxia caused tachycardia in

the dog, but the reflex action of carotid body chemoreceptors antagonized this effect, since chemoreceptive activation causes bradycardia [de Burgh Daly & Scott (130)]. The injection of potassium cyanide into the pulmonary circulation of the dog potentiated the bradycardic response to aortic nerve stimulation, and was thought to be mediated via the chemoreceptors [Rudomin Z. & Rubio (131)]; similar evidence for such chemoreceptors was also found in the rabbit [Rudomin Z. *et al.* (132)].

Respiratory arrhythmia in the dog, caused by variations in vagal tone of central origin, was influenced by degree and timing (in relation to respiratory phase) of baroreceptive afferent activity [Koepchen & Thureau (133)]. Tachycardia arising from decreased vagal tone in the dog, evoked by atropine, pentobarbital, or sodium cyanide, was accompanied by disappearance of respiratory arrhythmia while tachycardia caused by epinephrine administration was not [Russek (134)]. In diving snakes, the heart rate slowed markedly during diving, the ECG showing a prolongation of the P-R interval by 15 per cent, of the R-T interval by 130 per cent, of diastole by 30 times, and a pronounced T-wave shift [Johansen (135)].

Nervous control of myocardial function has been further elucidated. Tachycardia, increased force of myocardial contraction, and more rapid pressure rise and ejection of the left ventricle of the dog, similar to changes appearing during muscular work, were produced by electrical stimulation of the cardiac sympathetic nerves or the hypothalamus [Rushmer *et al.* (136)]. Pronounced changes of myocardial function occurred during introduction of anesthesia, intubation, artificial respiration, and thoracotomy. The response to muscular exercise could not be mimicked completely by intravenous infusion of catecholamines, partly because of interfering reflexes from the arterial system. Myocardial performance was further studied in human subjects and in dogs in relation to body position and activity [Rushmer (137)]; heart volume and stroke volume were smaller when the subject was sitting or standing than during recumbency and increased only slightly during exercise, not exceeding recumbent control values. The pronounced influence of stimulation of the sympathetic cardiac nerves on cardiac performance was similarly demonstrated by Kelso & Randall (138). During oligemic hypotension in dogs, the sympathetic nervous outflow was activated, resulting in increased myocardial force of contraction, increased concentration of plasma epinephrine and, to a small degree, of norepinephrine; eventually, in the acidotic terminal stage a lowered cardiac sensitivity to injected norepinephrine and a decrease in force of contraction developed [Walton *et al.* (139)]. In conclusion, these reports (136 to 139) demonstrate the quantitative importance of the cardiac sympathetic tone for myocardial performance under variable normal and pathological conditions in the intact animal or in man. Several other factors are undoubtedly in play, among them the property of cardiac fibres which increases force of contraction with greater fibre length, but apparently they are mostly overshadowed by the effect of sympathetic tone.

A clinical condition characterized by a high cardiac output ("hyperkinetic circulation") at rest as well as during muscular exercise in the absence of heart disease or any known cause was accompanied by proportional changes of the S-T and T regions of the ECG, which Holmgren *et al.* (140) suggested were "sympathicotonic". The hyperkinetic state, as well as the ECG changes, was corrected by systematic physical training. A similar type of high output state was accompanied by ECG changes suggestive of left ventricular hypertrophy [Gorlin *et al.* (141)]. "Sympathicotonic" ECG changes were frequent in patients with "nervous heart symptoms" [Friese & Haid (142); Hofmann (143)]. These observations (140 to 143) demonstrate that in-co-ordination of the neurohumoral control of the human circulatory system may develop and constitute an important factor for the cardiac patient's symptoms and signs.

*Visceral circulation.*—Sympathectomy affecting the gastric circulation in the rat produced increased mucosal blood content [Arabehty *et al.* (149)]. Sympathetic blockade by means of high spinal anesthesia resulted in an increase of renal blood flow, in relation to arterial blood pressure, and of diuresis; as the glomerular filtration rate remained unchanged, the increased diuresis was ascribed to a decreased tubular reabsorption [Langston *et al.* (150)].

*Cutaneous and muscular circulation.*—Measurements of peripheral blood flow in human subjects were made by modified methods for photoelectric plethysmography [Rawson (151)] and occlusion plethysmography [Graf & Westersten (152)], and thyroid blood flow was measured by a modification of Hensel's thermal conductivity method [Mowbray (153)].

Electrical stimulation in the cat's hypothalamus of the sympathetic cholinergic vasodilator outflow to skeletal muscle resulted in a pronounced increase of arterial inflow and venous outflow in the gastrocnemius muscle without change of local tissue clearance of  $\text{NaI}^{131}$  or  $\text{Na}^{24}\text{Cl}$ , suggesting functional separation of "shunt" and "nutritional" blood flows in muscle [Hyman *et al.* (154)]. Sympathetic blockade resulted in hyperemia but a decreased oxygen tension of leg skin in normal subjects, consistent with decreased capillary blood flow in spite of increased shunt flow [Davis & Greene (155)].

Resting forearm blood flow, measured by strain gauge plethysmography, increased with age in male subjects [Hellon & Clarke (156)]. The postural decrease of the volume-pulse amplitude of extracranial circulation was more pronounced in old than in young healthy men and differed in a group of coronary patients from the decrease in their control age group [Simonson (157)]. Sympathetic blockade produced dilatation in toe vessels in a group of hypertensive patients with fewer advanced retinal and renal vascular lesions than a second group of patients in whom no dilatation was obtained [Caliva *et al.* (158)]. Insulin hypoglycemia in young healthy subjects evoked a short initial decrease followed by an increase of skin blood flow and an increase of muscle blood flow, measured by the thermal conductivity method [Allwood *et al.* (159)]. The muscular vasodilatation could not be wholly reproduced by intra-

venous epinephrine infusion. When venous occlusion plethysmography was used, similar results were obtained in insulin hypoglycemia [Allwood & Ginsburg (160)]; local intra-arterial injection of atropine was without influence on resting blood flow but diminished the vasodilatation during hypoglycemia in the forearm, but not in the hand, suggesting that sympathetic cholinergic vasodilator fibres were activated in muscle.

During emotional stress in healthy students, the blood flow through the forearm increased, but did not change in the hand; this response was reduced by injection of atropine or nerve block and was smaller or absent in the sympathectomized arm of patients, also suggesting that cholinergic vasodilator fibres in muscle can be activated [Blair *et al.* (161)]. Muscular vasodilatation during mental arithmetic was found by Fencel *et al.* (162), while the occasional vasodilatation in the hand during such intellectual-emotional stress occurred only with marked sweating [Allwood *et al.* (163)].

In subjects with a "hyperkinetic" circulation (140), forearm blood flow was higher than in the control group, both at rest and during work on a bicycle ergometer while hand blood flow did not differ [Graf & Ström (164)]. Not until very low values of hemoglobin concentration (below 4 gm. per 100 ml.) were attained in anemia, did blood flow through the forearm increase; venous oxygen saturation in forearm vein blood was then found to be under 15 per cent [Verel & Duff (165)]. Cardiac output in anemia was earlier found to increase when the hemoglobin concentration fell below 8 gm./100 ml. Noise stimuli evoked reductions of hand volume and skin temperature which were variable between individuals [Oppliger & Grandjean (166)].

Peripheral vasodilatation evoked reflexly from thoracic receptors was reported and reviewed by Kramer (29). Mechanical stretching or positive-pressure inflation of the lungs in open-chest experiments on dogs produced peripheral vasodilatation and hypotension when peripheral circulation was effected by a heart-lung machine at constant flow rate; vagus blockade prevented the effect [Salisbury *et al.* (167)]. Spontaneous breathing against positive pressure (+15 mm. Hg) caused forearm vasoconstriction, while negative-pressure breathing caused vasodilatation in normal subjects; hand blood flow and distensibility of low-pressure capacity vessels in forearm and hand were not influenced [Blair *et al.* (168)]. Peripheral vasoconstriction was observed after the induction of anesthesia in cardiac patients and was followed by vasodilatation when the thorax was opened and the heart-lung machine started [Graf *et al.* (169)]. Deep inspiration was accompanied by changes in hand volume and venous blood pressure suggesting constriction of both resistance and capacitance vessels [Thron *et al.* (170)]; a constriction was also observed during hypoxia [Hintze & Thron (171)].

Mental arithmetic was the procedure studied which most effectively evoked a pressure increase in isolated venous segments under forearm skin, corresponding to venous vasoconstriction [Martin *et al.* (172)]. Spontaneous variations of venomotor tone were similar in subjects with and without congestive heart failure [Quiroz *et al.* (173)].

*Other organs.*—The pressure within the marrow of the cat's femur amounted to 114 to 124 mm. Hg in different animals, varied passively with the arterial blood pressure, decreased upon stimulation of the abdominal sympathetic trunk or injection of epinephrine or norepinephrine and increased after sympathetic denervation [Herzig & Root (174)]. The plasma concentration of non-esterified fatty acids increased during orthostatic testing in normal subjects and in an adrenalectomized patient but remained constant in a patient with primary autonomic insufficiency, indicating that the sympathetic innervation of fat tissue partakes in the mobilization of fatty acids [Hamlin *et al.* (175)].

Stimulation of baroreceptors by carotid sinus distention in the cat provoked inhibition of the tonically active gamma motoneurons in the L<sub>7</sub> ventral root but not of alpha motoneurons, suggesting that baroreceptor stimulation suppresses the somatic activity of the reticular activating system or activates the reticulospinal suppressing system [Schulte *et al.* (176)]. Efferent stimulation of the sympathetic trunk in the cat produced first a rise, then a fall in excitability of muscle spindle receptors (but not of tendon organ receptors), as evidenced by changes in the amount of stretch required to alter the discharge frequency in spindle afferent fibres, and by their response to gamma motor stimulation [Hunt (177)]. A similar effect was induced by intravenous injection of epinephrine. The response was obtained even during arrest of circulation to the muscle studied. Eldred *et al.* (178) observed a similar effect of efferent sympathetic stimulation on the afferent firing of spindles and of tendon organ afferents, but concluded that the effect was secondary to vasoconstriction.

#### NEUROENDOCRINE SYSTEM

*Adrenal medulla.*—Electrical stimulation of the posterior hypothalamus with increasing intensity, frequency, or duration produced first a neural sympathetic discharge (contraction of the innervated nictitating membrane), then adrenomedullary discharge (contraction of the denervated nictitating membrane) [Gellhorn (179)]. Electrolytic lesions in the posterior hypothalamus of the dog were followed after a few days by increased contents of epinephrine and norepinephrine in the adrenals while after lesions in the anterior hypothalamus the norepinephrine content was decreased [Tigyi *et al.* (180)]. Activation of the sympathetic vasodilator pathway in the cat's medulla also led to increased adrenomedullary output, epinephrine increasing more than norepinephrine [Lindgren *et al.* (181)]. The secretion rate of catecholamines might reach the threshold for systemic vascular reactions, at least during central stimulation of high intensity [Lindgren *et al.* (182)].

Epinephrine and norepinephrine concentrations in human plasma were determined by a modified spectrofluorometric method during a variety of conditions in normal subjects and in patients; the resting concentration of epinephrine in venous plasma was 0.07 µg./l. and of norepinephrine 0.35



$\mu\text{g./l.}$ ; an increased concentration of norepinephrine was found during muscular exercise of high intensity [Vendsalu (183)]. The half life of infused norepinephrine in plasma was 2.3 min., and the normal resting secretion of this substance was calculated as 0.018  $\mu\text{g. per kg. min.}$ , of epinephrine as 0.008  $\mu\text{g. per kg. min.}$  These values are in reasonable accord with data of Cohen *et al.* (184). The urinary excretion rate of norepinephrine but not of epinephrine was depressed by long-term reserpine treatment in schizophrenic patients [Carlsson *et al.* (185)], while no such effect was obtained by iproniazid treatment in depressive patients [Carlsson *et al.* (186)].

Insulin hypoglycemia in the rabbit caused the appearance, at a certain critical level of plasma glucose concentration, of an epinephrine-like substance which did not come exclusively from the adrenals [Armin & Grant (187)]. The catecholamine output in adrenal venous blood in dogs was increased above "resting" level during preoperative procedures causing excitement, pain, discomfort, or tissue trauma and was lowered by barbiturate anesthesia [Walker *et al.* (188)]; blood loss produced an immediate increase of the output of epinephrine and, to some extent, of norepinephrine [Walker *et al.* (189)]. Catecholamine output from the adrenals of the cat increased after ethanol doses above 0.60 gm. per kg. [Perman (190)], and roentgen-ray irradiation of the whole body of rabbit, rat, and cat decreased the catecholamine content of the adrenals [Goodall & Long (191)].

Thyroid administration in mice produced a decrease of the histochemically identified norepinephrine content of the adrenal medulla, while thyroidectomy or thiouracil administration increased it [Hopsu (192)]. Administration of ACTH produced adrenocortical hypertrophy but no change of the adrenal medulla, while somatotropin caused medullary hypertrophy. These effects of hormonal influence were not influenced by splanchnic nerve section, which by itself caused a moderate atrophy of the adrenal medulla.

Adrenalectomy in patients with breast cancer or diabetes, with cortisone substitution, decreased urinary catecholamine excretion variably but did not influence the circulatory reaction or the blood lactate increase during muscular work; the resting blood volume and heart volume were also not affected [Birke *et al.* (193)]. Acute anoxic anoxia in dogs caused a rise in heart rate and stroke volume and a fall in arteriovenous oxygen difference and peripheral resistance; these effects could be partly reproduced in a control dog by transfusion of blood from such anoxic dogs and were at least partly prevented by adrenalectomy [Baugh *et al.* (194)].

The relaxing effect of epinephrine on rabbit intestine was associated with increased lactic acid production [Mohme-Lundholm (195)]. Epinephrine added *in vivo* or *in vitro* inhibited the amino acid uptake of rat diaphragm [Wool (196)]. The glycogenolytic effect of epinephrine on muscle glycogen was estimated to be at least five to ten times as potent as on liver glycogen in rats [Sokal & Sarcione (197)].

The glycogenolytic effect of epinephrine on frog liver was accompanied by

lactic acid production, acidosis, and a loss of potassium [Craig (198)]; these effects were counteracted by dihydroergotamine and dibenamine [Craig & Mendell (199)]. Acceleration of blood coagulation was observed after pain stimulation, and Markosian (200) suggested that this was dependent on reflex epinephrine secretion; a similar response to acute stress in rats was not influenced by adrenalectomy, however [Friedman & Uhley (201)].

*Adrenal cortex.*—Stimulation of the amygdaloid nucleus activated, while stimulation of the hippocampus inhibited, ACTH secretion in different animals [Endröczy *et al.* (202)]. Bilateral lesions in the medial hypothalamus, notably within the ventromedial and dorsomedial nuclei, diminished the urinary excretion of 17-hydroxycorticosteroids in guinea pigs [Winkler *et al.* (203)]. Similarly placed lesions in rats led to adrenal atrophy [Moll (204)]. Signs of neurosecretory activity in the supraoptic and paraventricular nuclei in rats did not correlate well with ACTH secretion when the latter was stimulated or inhibited [Kivalo & Rinne (205)]. Acute blood loss in dogs resulted in diminished adrenal blood flow, increased venous concentration, and constant or increased output of corticosteroids [Walker *et al.* (206, cf. 188, 189)].

Administration of cortisone and different corticosteroid esters intravenously was reported to produce measurable cardiovascular effects in normal subjects and patients [cf. (110), however]; an increase of cardiac stroke volume and a decrease of peripheral resistance were reported by Hoffmann & Emmrich (207), while a decrease of stroke volume and an increase of peripheral resistance were reported by von Kügelgen *et al.* (208). The arterial pressure increase in response to epinephrine and angiotensin was augmented by DCA or metacorticoid treatment, but the vasoconstrictor response in the perfused hindlegs of the rat remained unaltered [Carlini *et al.* (209)]. Adrenalectomy in rats caused lowered excitability of peripheral nerve, apparently secondary to sodium depletion [Wright & Lester (210)].

*Aldosterone secretion.*—Hypophysectomy in dogs with hyperaldosteronism caused by experimental ascites was followed by a pronounced decrease of both aldosterone and corticosterone secretion rates [Davis *et al.* (211)]; hypothalamic lesions thereafter did not cause any consistent further change. When primary hypothalamic lesions were made, a decrease of aldosterone secretion and adrenal atrophy occurred only after destruction of the median eminence [Davis *et al.* (212)]. Denervation of the carotid sinus or vagotomy did not influence aldosterone secretion [Davis *et al.* (213)]. The rate of urinary aldosterone excretion in normal subjects rose during prolonged standing, with a decreased output of both sodium and potassium [Gowenlock *et al.* (214)].

*Pituitary and ovarian hormones.*—Separate release of human oxytocin during suckling and of antidiuretic hormone during hypertonic infusion could be attained [Theobald (215)]. Intravenous administration of oxytocin caused peripheral vasodilatation in the human subject, but this effect was abolished by 1/20 the unit amount of vasopressin [Kitchin *et al.* (216)]. Ovariectomy in rats produced, and estradiol treatment prevented, a decrease of myo-

cardial force of contraction and actomyosin content [King *et al.* (217)]. A vascular constrictor and pressor response to oxytocin, which normally has vasodilator action, and an increased pressor response to vasopressin were observed in female rats during estrus and in the latter half of pregnancy, and in male rats during treatment with progesterone and stilbestrol, while ovariectomy had an opposite effect [Lloyd (218, 219)]. The sensitivity to acetylcholine was not influenced. The vascular sensitivity to norepinephrine in dogs was not influenced by ovariectomy [Zsotér *et al.* (220)]. After ovariectomy in rats, estradiol treatment produced an increased resting potential of the single uterine fibre, with rhythmical spontaneous contractions accompanied by a train of action potentials; acetylcholine depolarized, stimulated contractions, and increased action potential frequency while epinephrine had the opposite effect [Marshall (221)].

Hypophysectomy in rats was followed by decreased metabolic rate, decreased heart weight and blood volume, bradycardia, a small decrease of stroke volume, a small increase of peripheral resistance, and a decrease of arterial pressure and cardiac output [Beznák (222, 223)]. The primary cause was judged to be the decreased demand for oxygen transport since treatment with thyrotropin prevented the circulatory changes. When cardiac work was increased by aortic constriction, left ventricular hypertrophy occurred when somatotropin was administered but not when only thyrotropin was given; on the other hand, the thyrotropin-treated animal's heart showed higher force of contraction per unit of myocardial weight.

In rats with unilateral nephrectomy and a high salt intake, constant stress combined with somatotropin treatment was most effective in producing hypertension and renal and cardiac lesions [Hall & Hall (224)].

In dogs, hypophysectomy did not produce an arterial pressure fall below the normal level but prevented the hypertension otherwise caused by carotid sinus constriction [Hawthorne & Gaspar (225)].

#### GASTROINTESTINAL SYSTEM

*Smell and taste.*—Natural stimulation of the rabbit's olfactory mucosa induced a slow receptor potential similar to that in the frog [Ottoson (227)]. In the frog, the slow olfactory-bulb potential which closely followed the olfactory receptor potential was found to be synaptic in nature [Ottoson (228)], and it could be differentiated from the postsynaptic response of the second order neuron [Ottoson (229)]. In the chorda tympani of rhesus monkeys the taste fibres were usually specific, and their receptors responded to one of the four classes of salt, acid, quinine, and sugar, but in some cases also to distilled water [Gordon *et al.* (230)]. In chickens, the taste receptors responded to salt, acid, quinine, and water but not to sucrose, while in pigeons, responses were obtained to salt, acid, and water (and to saccharine) [Kitchell *et al.* (231)].

*Salivary glands.*—Stimulation of the cranial end of the cut cervical vagosympathetic trunk in dogs caused secretion from the parotid gland; the

effect was thought to be transmitted via efferent sympathetic fibres [Langley & Smith (232)]. During operations, the human chorda tympani was stimulated electrically, which caused a secretion of saliva from the sublingual duct [Diamant *et al.* (233)]. Parotid secretion was reflexly evoked in sheep and calves by mechanical distention of the esophagus, while it was inhibited by distention of the rumen [Kay & Phillipson (234)].

*Stomach.*—Release of gastrin by reflexly evoked vagal activity was demonstrated in experiments on dogs with Heidenhain pouch or transplanted corpus pouch and with the innervated pyloric antrum isolated from the gastric canal [Maung pe Thein & Schofield (235)]. Sham feeding produced a large acid secretion in the Heidenhain pouch and a definite secretion in the corpus pouch; the secretion was inhibited by acidification of the antral pouch, and it was abolished after vagal denervation of the antrum. Apparently, acidification of the antrum suppressed the vagal release of gastrin. The release of gastrin was independent of antral motility.

After cervical spinal cord section and bilateral vagotomy in dogs, gastric secretion was not immediately stimulated by the introduction of food into the stomach, but stimulation occurred later when the food constituents became hydrolyzed [Popov & Sokolskaia (236)]. Afferent abdominal vagal stimulation resulted in reflex depression of gastric motility and stimulation of gastric secretion of acid and pepsin, and sometimes in pancreatic amylase secretion, the latter response being similar to the effect of efferent vagal stimulation [Harper *et al.* (237)].

Brain surgery was followed in one patient by paralytic ileus, in another by acute gastric erosions and perforation [Arseni & Samitca (238)]. Electrical stimulation of the "sympathetic division" of the cat's hypothalamus caused, besides sympathetic activation, inhibition of intestinal motility, which was followed by poststimulatory "rebound" activation; these effects persisted after vagotomy [Gellhorn (239)]. During stimulation, bladder motility was usually stimulated even when intestinal motility was inhibited unless considerable adrenomedullary activation took place [Gellhorn (240)]. Gastric hunger contractions were similar in normal rats and in rats made hyper- or hypophagic after hypothalamic lesions; the contractions were similarly inhibited by heat, cold, and epinephrine injection, but in the hyperphagic rat glucagon did not produce ordinary inhibition [Mayer & Sudsaneh (241)].

The excitatory effect of low-frequency efferent vagal stimulation on gastric motility in the cat was potentiated for up to seven minutes by a preceding period of high-frequency stimulation; such posttetanic potentiation was also observed in intestinal motility [Blair *et al.* (242)]. Distention of the rumen in conscious sheep inhibited local motility and also inhibited parotid salivary secretion [Ash & Kay (243)]. Light tactile stimulation of the esophagus, cardia, and reticulorumen fold accelerated reticulum contractions and caused rumination. Stimulation of rumen contractions was evoked by

stretch of the reticulum and reticulorumenal fold, and inhibition by rumen distention, in decerebrate sheep and goats [Titchen (244)]. The reticulum contained larger amounts of cholinesterase than the rumen or abomasum in sheep [Brunaud *et al.* (245)].

*Pancreas and intestines.*—Pancreatic secretion with output of amylase was provoked in rats by moderate insulin hypoglycemia or by efferent vagal stimulation during secretin facilitation; the hypoglycemic response was abolished after vagotomy [Lin & Alphin (246)]. Low-frequency stimulation of the sympathetic outflow to the intestines in the cat produced a rapid vasoconstriction and a late inhibition of motility; inhibition could also be evoked by mechanical obstruction of the artery and was ascribed to reduced local blood flow [Celander (247)]. High-frequency sympathetic stimulation sometimes caused rapid inhibition of motility, attributed to local transmitter "overflow". Intestinal inhibition in the cat, evoked either reflexly by carotid artery occlusion, withdrawal of blood, or stimulation of somatic afferent nerve fibres, or directly by stimulation of the sympathetic outflow, was dependent on intact adrenal glands and was attributed to release of adrenomedullary catecholamines [Kock (248)]. Even after sympathectomy, a local inhibition of intestinal motility occurred as a prompt response to distention of a neighboring part of the intestine. In conclusion, in these reports (247, 248) no evidence was found for the existence of specific sympathetic inhibitory fibres in the intestinal muscular wall, but such factors as adrenomedullary secretion, reduction of local blood flow, and vasoconstrictive transmitter overflow, in decreasing order of importance, appear responsible for centrally relayed reflex intestinal inhibition (247, 248).

The changes of tonus and motility in isolated segments of rabbit intestine, which occurred spontaneously or after addition of epinephrine, were accompanied by corresponding changes in acetylcholine release from the intestine into the surrounding fluid, suggesting that spontaneous motility, as well as the action of epinephrine, depends on acetylcholine metabolism [Tidball (249, 250)]. The acetylcholine content of the guinea pig ileum decreased immediately after stretching of the wall and returned rapidly after release of the stretch [Hayama & Ikeda (251)].

*Afferent pathways and reflexes.*—Afferent volleys from the splanchnic sympathetic nerves were recorded in the cerebellar cortex, most regularly in the lobulus simplex [Bratus (252)], and in thalamic nuclei [Delov *et al.* (253)]. Afferent stimulation of the abdominal vagus, or distention of the esophagus or stomach, provoked arterial pressure increase, gastric relaxation, and inhibition of the patellar reflex, in rabbits and cats [Cragg & Evans (254)]. Single-shock afferent stimulation of splanchnic and pelvic nerves caused first facilitation, later depression of the monosynaptic lumbosacral spinal reflex in the cat [Evans & McPherson (255)]. Single-shock afferent stimulation of the splanchnic nerve also produced facilitation and discharge of motoneurons to intercostal muscles in the rabbit; an early discharge evoked by afferent A

fibres was seen, followed by a late discharge evoked by afferent C fibres [Alderson & Downman (256)]. Reflex contraction of paravertebral muscles was also observed in response to visceral stimulation [Eble (257)].

*Control of vomiting.*—The emetic response to cerebral intraventricular injections of epinephrine and apomorphine in unanesthetized cats was abolished after ablation of the area postrema; oral administration of copper sulphate evoked vomiting even after such ablation, demonstrating that a co-ordinated response could take place [Borison (334)]. Similar ablation in dogs increased the time for appearance of vomiting after bilateral nephrectomy or after intravenous injection of guanidine [Borison & Hebertson (335)]. The sensitivity of the vomiting response to lanatoside-C injection or to total-body roentgen irradiation increased markedly with increasing age and weight in cats and kittens; no vomiting was observed in kittens below 250 gm. body weight (10 days of age) [Brizzee & Vitale (336)].

#### GENITOURINARY SYSTEM

*Kidney.*—Lesions in the paraventricular nuclei in rats caused a threefold increase of urinary sodium excretion but no change of potassium excretion during the first ensuing 24 hours, independent of adrenalectomy or renal denervation [Keeler (258)]. The urinary excretion rate of sodium in the dog was increased by activation of the low-pressure volume receptors of central circulation [Pabst & Gauer (259)]. A decrease of the thoracic blood volume in the dog, caused by hemorrhage or by positive-pressure breathing, produced an increase of circulating level of antidiuretic hormone and appeared to dominate over competitive osmoregulation; the response to fluid loading or negative-pressure breathing was less dominant [Baratz & Ingraham (260)]. Standing up caused sodium retention in man with an increased hydrogen ion excretion and a slowly increasing potassium excretion, while lying down induced reverse changes [Thomas (261)].

*Bladder.*—Contraction and inhibition of the bladder were evoked from two distinct regions in the lateral reticular formation of the cat's medulla [Tokunaga & Kuru (262)].

Stimulation of the nervous outflow to the cat's bladder showed an optimal effector summation at a stimulation rate of 10 to 15 per second; the summated plateau of contraction fell at lower stimulation rates [Carpenter & Tankersley (263)]. A sustained flexor reflex evoked by bladder distention in spinal and decerebrate cats depended in part on gamma-motor activation but was still present after deafferentation of the participating muscles [Abdullah & Eldred (264)]. Bladder distention in the cat depressed the ventral root monosynaptic reflex [Evans & Mc Pherson (265)]. Under chloralose anesthesia, the flexor reflex was also depressed; but in decerebrate cats moderate bladder distention enhanced the flexor reflex, while a few hours after spinal transection strong distention was required to produce only slight enhancement.

*Gonads.*—Hypothalamic lesions just caudal to (but not lesions rostral to)

the optic chiasma produced earlier puberty in female rats, with vaginal canalization, increased uterine weight, and ovulation, and in some cases prolonged estrus with absence of corpora lutea in the ovaries; the lesion was believed to cause release of pituitary gonadotropic secretion from inhibitory hypothalamic influence [Donovan & van der Werff ten Bosch (266)]. Obesity was not produced by the lesion. Similar lesions in the ferret hastened the onset of estrus and caused depression of thyroid iodine uptake; chronic stimulation of this hypothalamic region did not cause release of gonadotropin [Donovan & van der Werff ten Bosch (267)]. In the rat, too, precocious ovarian stimulation was produced by lesions in the anterior hypothalamus [Elwers & Critchlow (268)], but lesions in the medial amygdaloid were also effective. The most effective lesion producing prolonged estrus without corpora lutea in rats was localized immediately above and slightly caudal to the chiasma, while lesions immediately dorsal to the paraventricular nuclei evoked prolonged diestrus with an abnormally large number of corpora lutea [Flerkó & Bárdos (269)]. In the hen, on the other hand, hypothalamic lesions caused an interruption of ovulation [Ralph & Fraps (270)].

#### OTHER NEUROEFFECTOR SYSTEMS

*Sweat glands.*—Methods for collection of sweat from the human arm [Barrueto *et al.* (271)], for quantitative measurement of sweating by a modified triketohydrindene hydrate (Ninhydrine) method [Pontén (272)], and for continuous measurement of sweating [Nakayama & Takagi (273)] were described. The water loss of dogs in hot and dry surroundings was not influenced by atropine or pilocarpine, suggesting that cholinergic sweating was of little or no importance [Koenig *et al.* (274)]. The response of eccrine sweat glands in the human forearm to indirect body heating was depressed by local injection of acetylcholine in high concentration; prolonged sweat gland activity did not precede the depression, and "desensitization" to the natural transmitter rather than "fatigue" was suggested as an explanation [Collins *et al.* (275)]. Prolonged arterial occlusion also depressed the sweat gland activity in the forearm and decreased the sensitivity to intradermal acetylcholine [Collins *et al.* (276)].

In patients with spinal cord lesions, thermoregulatory sweating was absent in skin areas with preganglionic sympathetic denervation [Boshes & Blustein (277)], but "spinal reflex sweating" in denervated areas, usually together with somatic and visceral hyperactivity, was observed for several years after the lesion. After transplantation the grafted skin regained nervous control of sweating if and when somatic sensory reinnervation became apparent [Pontén (278)]. Reinnervated sweat glands showed hypersensitivity to electrophoretic stimulation with nicotine. Sebaceous gland function, on the other hand, was independent of sensory or sudomotor reinnervation.

*Electrical skin resistance.*—The galvanic skin reflex at the palm and dorsum of the hand was depressed at low temperatures of the room and of the



skin, and enhanced at high temperatures [Yokota *et al.* (279)]. Afferent stimulation of a cutaneous nerve evoked a galvanic skin reflex which was followed by a long lasting depression of a repeated test reflex; the depression was judged to be supraspinal, as it almost entirely disappeared after spinal cord section [Wang & Hind (280)]. The psychogalvanic response in human subjects to cutaneous stimulation by thermal radiation was larger and of longer duration if a stimulus of given intensity evoked pain sensation, and it was therefore judged to be an accurate discriminator of subjective pain sensation [Mc Kenna (281)].

*Spleen.*—Splenic nerve stimulation or epinephrine infusion decreased inflow and increased outflow of blood in the dog's spleen, and its weight diminished [Green *et al.* (282)]; adrenergic blockade decreased this effect. Acetylcholine increased inflow, outflow, and weight; atropine abolished this effect. The splenic contraction in response to hypercapnia in dogs was mediated mainly by adrenomedullary secretion but also by splenic nerve activity [Ramlo & Brown (283)]. Venous blood from the spleen, especially after splenic nerve stimulation, exerted a positive inotropic effect on the dog's heart-lung preparation [Braasch & Schmier (284); Alella *et al.* (285)]. A similar effect was obtained from venous blood from the liver with the spleen intact. The positive inotropic effect of ox spleen extract on the guinea pig's heart was not abolished by dichloroisoprenaline in doses that blocked the inotropic effect of norepinephrine [Corbin & Thorp (286)]. As catecholamines could not be chemically identified in the spleen extract, the inotropic effect of the extract was attributed to other factors.

*Sympathetic denervation.*—Local sympathectomy or reserpine treatment in the cat was followed by a decrease in the norepinephrine content of the spleen and of the iris of the eye, by a corresponding increase in the sensitivity to injected norepinephrine, and by a decrease in the effect of tyramine [Burn & Rand (287)]. The action of nicotine on the pilomotor activity in the cat's tail, on the cat's nictitating membrane, and on the vessels of the perfused rabbit ear diminished or disappeared after sympathetic denervation or reserpine treatment [Burn *et al.* (288)]. As such treatment also resulted in a decrease of norepinephrine and chromaffin cells in the examined organs, it was concluded that nicotine acts on sympathetic nerve fibres with resulting liberation of norepinephrine from peripheral stores which may be the chromaffin cells.

#### TEMPERATURE REGULATION

*Receptors and afferent systems.*—Cold receptors were present in the cat's leg skin and fired at a maximal rate around 30°C. but were also sensitive to local mechanical stimulation [Witt & Hensel (289)]. Mechanoreceptors were not wholly specific either, and they increased their firing rate with increasing local temperature. Warm receptors were not demonstrated. Temperature-sensitive receptors with afferent fibre conduction velocities of 0.5 to 1.2 m./sec. were found in the cat's hindleg skin; they were relatively insensitive

to mechanical stimulation [Iggo (290)]. Temperature-sensitive (cold) receptors in the cat's hindleg skin with afferent C fibres were demonstrated by Douglas *et al.* (291) in a similar way. They were also mechanosensitive.

Cold receptors in the cat's tongue were activated by decrease of local temperature regardless of slope or direction of the tissue's spatial temperature gradient [Hensel & Witt (292)]. Cold receptors but no warm receptors were found in the tongues of chickens and pigeons [Kitchell *et al.* (231)]. The activity of single neurons, in the thalamic nucleus ventralis posteromedialis of the cat with afferent connections from the tongue, was studied by Landgren (293). Touch and cold reception were most commonly represented.

*Central regulation.*—Conductive heating of the anterior hypothalamus in the unanesthetized cat produced the expected change of body posture, a rise of pad temperature, and suppression of shivering but no panting, while conductive cooling produced the reverse postural change and a moderate fall of skin temperature below control level but no shivering [Freeman & Davis (294)]. In acute experiments on anesthetized cats, heating of the anterior hypothalamus was followed by a rise of skin temperature and a moderate fall of rectal temperature (in 23 of 37 trials), while hypothalamic cooling was not followed by any skin temperature change or any significant change of rectal temperature (a slight rise in 13 of 37 trials and a fall in 6).

Hypothalamic conductive cooling in the unanesthetized cat produced a diminution of the ear blood flow at indifferent room temperature but not in warm surroundings when the ear vessels had dilated [Krüger *et al.* (295)]. In the unanesthetized dog, conductive cooling of a relatively large hypothalamic region via needles produced shivering and a fall of skin temperature [Hammel *et al.* (296)]; the body temperature increased during continued cooling, and alternating cooling and heating caused alternating shivering and panting, sometimes overlapping in time. Warming of the rat's brain caused coordinated thermoregulatory responses [Donhoffer *et al.* (297)]; after chronic electrolytic lesions in the hypothalamus, long-term increases as well as decreases of basal metabolic rate and body temperature were observed [Mestyan *et al.* (298)]. Cooling of the entire dog's brain via the blood stream, while body and skin temperatures were kept constant, caused a progressive decrease of oxygen uptake, respiratory frequency, and tidal volume, and of arterial pressure and peripheral resistance, while cardiac stroke volume, frequency, and output were uninfluenced or showed moderate variable changes [Brendel (299 to 301)]. The animals were anesthetized with chloralose and subjected to relatively extensive operative procedures, including ligation of vertebral and spinal arteries, but additional experiments on unanesthetized dogs corroborated the general result. Skin cooling, on the other hand, evoked reflex shivering and increased oxygen uptake; when the brain temperature was continuously decreased, the reflexly evoked shivering was successively depressed and disappeared at a brain temperature of about 30°C.

According to the conclusions drawn in these reports (294–301), cooling of the anterior hypothalamus appears to produce adequate responses from some

of the thermoregulatory effector organs, including cutaneous vasoconstriction, at least when the cooling affects a sufficiently large region and is performed in the warm, unanesthetized animal. Species differences undoubtedly involve differences in the response pattern of the different effector organs or mechanisms. The evidence regarding centrally induced shivering is still contradictory; it apparently can be evoked under certain circumstances.

*Efferent pathways and reflex control.*—An abrupt rise of environmental temperature may cause panting in sheep without any initial change of deep body temperature [Bligh (302)]. Such reflex panting originated from thermoreceptors both in general body surface and in the nasobuccal area. The frequency spectrum of shivering in man contained both rhythmical and arrhythmical components of larger amplitude than in voluntary contraction [Lippold *et al.* (303)]. In cats a similar result was found. The rhythmical component was apparently caused by self-oscillation in the reflex loop, as it disappeared after deafferentation. The cold-induced increase of oxygen uptake in rats (which was "centrally evoked") could be partly blocked by body diathermy, which did not affect the other part apparently caused by skin cooling ("peripherally evoked") [Davis *et al.* (304)]. The peripherally evoked part resulted chiefly from shivering before acclimatization to cold, but during acclimatization shivering disappeared; the centrally evoked part remained unaltered. The "neutral zone" value of the integrated skin temperature of newborn babies was considerably higher than in adults [Brück (306)]. At a normal central body temperature, cutaneous vasodilatation occurred at an integrated skin temperature of about 35.3°C. and perspiration at about 37.1°C., while a two- to threefold increase of the oxygen uptake was provoked when the surrounding temperature was lowered from the neutral zone of 32°–34°C. to 28°C. although skin temperature only fell to about 35°C.

About one-half of the femoral arterial blood passed through arteriovenous anastomoses in the dog's leg, but temperature changes of the arterial blood were almost totally equilibrated in one passage, indicating favorable heat exchange conditions [Piiper (307)]. Cold-induced vasodilatation was studied by means of finger plethysmography and calorimetry [Edwards & Burton (308)]. With vasoconstriction, the digital arterial temperature might fall as low as 14°C., and increased viscosity of blood probably added considerably to the reduction of blood flow. The sensations and responses to immersion of a hand in hot as well as cold water were diminished by habituation [Glaser *et al.* (309)]. Habituation was inhibited by chlorpromazine administration. Adrenalectomy impaired the maintenance of body temperature and the peripheral vasoconstriction during cold stress in hamsters [Wyman & Drapeau (310)]; cortisone therapy restored the responses. A modified thermal conductivity meter was used to study mucosal blood flow in the human tongue and nose [Demling *et al.* (311)]. Cooling produced an initial decrease followed by a secondary increase of flow.

The lowest rectal and average skin temperatures compatible with continuous sleep in cold surroundings (–33°C.) were 35.5 and 30.5°C., respec-

tively, in young men [Kreider & Iampietro (314)]; oxygen uptake was not higher than it was during sleep in warm surroundings. After acclimatization to cold, a lower rectal temperature was attained during sleep than before [Kreider *et al.* (315)]. Aborigines from central Australia showed a more rapid fall of body temperature, without metabolic compensation, caused by cold stress during sleep than white men, indicating less reaction to changes in body temperature [Hammel *et al.* (312)]. Timmerman *et al.* (313) studied the diurnal change of human body temperature and concluded that it was governed partly by environmental factors but also by intrinsic factors.

Moderate fever caused by respiratory tract infection did not influence the response pattern of rectal and skin temperatures and of sweating during heat stress in a subject [MacPherson (316)]. Intravenous injection of a purified bacterial pyrogen caused cutaneous vasoconstriction in the hand within one hour [Bryce-Smith *et al.* (317)]. After a moderate dose, the reflex cutaneous vasodilatation which normally occurs on radiant heating of the body could still be evoked. The constrictor response to pyrogen, as well as the reflex dilatation, was abolished by nerve block of the hand. The blood flow through muscles in forearm and calf was not influenced by experimental fever as long as shivering did not occur [Bock & Golenhofen (318)].

Rapid rewarming of dogs under pentobarbital from hypothermia of 20°C. or 25°C. did not usually provoke circulatory failure, but slow rewarming did [D'Amato *et al.* (319)], although apparently not because of myocardial insufficiency. During hypothermia in cats, the cardiac response to efferent vagal stimulation disappeared at a body temperature of 24°C., while the circulatory reflex response to afferent sciatic nerve stimulation, although diminishing below 30°C., did not disappear until 20°C. [Ziemlański & Markiewicz (320)].

During heat stress in unanesthetized dogs not given water, hypoglycemia appeared, apparently because of increased glucose utilization arising from increased respiratory work during panting [Kanter (321)].

The increase of human body temperature during standardized muscular exercise was considerably larger after a period of restricted food and water intake [Grande *et al.* (322)].

Cold diuresis in the dog also involved increased para-aminohippuric acid and inulin clearances and increased excretion rate of sodium, potassium, and chloride [Pabst & Thron (323)]. Cold diuresis in the rat was absent after hypophysectomy or adrenalectomy while thyroidectomy was without influence [Itoh *et al.* (324)]. On the other hand, thyroidectomy in the rat influenced the readjustment of body fluids in response to cold exposure, with a relatively greater water and sodium content of tissues than in intact rats [Boatman (325); Boatman *et al.* (326)].

#### REGULATION OF BODY FLUIDS AND BODY WEIGHT

*Body fluid regulation.*—The firing rate of single neurons within the supra-optic nucleus in the rabbit's hypothalamus was generally accelerated by the

intracarotid injection of hypertonic fluid, while neurons in the paraventricular nucleus were inhibited [Cross & Green (327)]. Neurons sensitive to osmotic stimulation appeared to be specific as they did not generally respond to tactile, auditory, or visual stimuli; other hypothalamic neurons responding to peripheral stimuli were not influenced by osmotic stimuli. Ablation of the supraoptic region of the hen's hypothalamus usually resulted in polydipsia while lesions in other parts of the hypothalamus failed to do so [Ralph (328)]. Histological and histochemical changes in the supraoptic and paraventricular nuclei in rats were observed after long-term excess salt administration, indicating hyperfunction, and after water administration, indicating hypofunction [Campanacci (329)]. Diminution of the total blood volume in dogs evoked oliguria and an increased urinary secretion of antidiuretic hormone; this effect was prevented by vagotomy [Lemaire (330)].

*Body weight regulation.*—Lesions in the pyriform lobe and the amygdaloid in the cat led to increased food intake and increasing body weight; neodecortication did not influence the effect [Morgane & Kosman (331)]. Hypothalamic lesions in the one partner of parabiotic rats, causing hyperphagia and obesity, produced hypophagia and thinning of the other partner, suggesting the existence of a negative feedback control of food intake [Hervey (332)]. If hypothalamic lesions were subsequently made also in the other partner, its hypophagia changed into hyperphagia.

Gastric motility during a standardized four-hour test was similar in obese and nonobese women, and no correlation appeared to exist between degree of obesity and gastric motility [Koch & Stunkard (333)]. A similar conclusion was drawn from experiments on rats (241).

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## HIGH NERVOUS FUNCTIONS: BRAIN FUNCTIONS AND LEARNING<sup>1,2,3</sup>

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The widespread recent use of chronically implanted electrodes for stimulation and recording in unanesthetized animals has made available a large amount of data relevant to the dynamic processes involved in the establishment and performance of conditioned responses. The volume and diversity of this information, which is broadly distributed throughout the international literature, seem to warrant an attempt to summarize and systematize recent findings in this domain. Rather than catalogue the many exciting reports which have appeared, we have undertaken to extract from these researches as detailed a view as we can provide about a number of more or less arbitrary phases which can be identified in the process of conditioned response elaboration.

The decision to devote this review to an evaluation of our current understanding of this process has necessitated a parallel rather than serial presentation of the individual researches. In view of the availability of excellent recent reviews of a more general nature (22, 81, 86) and the constraints of space, a number of elegant and exciting studies not directly relevant to this problem have been omitted. Ablation and drug studies have been discussed here only insofar as the results seem pertinent to the central purpose outlined above.

The phases of conditioned response elaboration which emerge from this body of research are (a) the response configuration of the experimentally naive animal to the initial presentation of a novel stimulus; (b) the alteration of this configuration on repeated presentation of that stimulus without associated reinforcement; (c) the effects of the initial introduction of reinforcement; (d) the appearance of the first conditioned response; (e) achievement of a terminal level of performance of the conditioned response; (f) differentiation of conditioned response to a reinforced and a nonreinforced stimulus; and (g) extinction of the conditioned response.

<sup>1</sup> This chapter is based on a survey of the literature which was completed in June 1960 and was prepared with the assistance of grants from the National Institute of Mental Health.

<sup>2</sup> Abbreviations used in this chapter include: CR (conditioned response); CS (conditioned stimulus); EEG (electroencephalogram); EMG (electromyogram); ERG (electroretinogram); RF (reticular formation); SD ([cortical] spreading depression); US (unconditioned stimulus).

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## RESPONSE TO A NOVEL STIMULUS

*Arousal.*—Presentation of a novel stimulus to an animal results in apparent alerting or arousal, accompanied by searching movements or orientation toward the source of the stimulus. The widespread low-voltage fast activity, or desynchronization, which is the characteristic electrographic sign of arousal, has frequently been interpreted as indicative of a generalized excitatory influence presumed to originate in the brainstem reticular activating system.

Apparently, the brainstem reticular formation (RF) is not indispensable for behavioral and EEG arousal. Adametz (1) reported that massive lesions in that structure, which resulted in a permanently comatose animal when made in one stage, did not abolish alertness when made in multiple stages. Lourie *et al.* (70) and Chow (23) have observed clear EEG desynchronization and behavioral arousal after massive reticular formation lesions inflicted in multiple stages.

Kogan (62) has used central threshold determinations to show that cortical desynchronization may be accompanied either by increased or decreased neural excitability. He observed that cortical desynchronization during a sensory stimulus was accompanied by a decrease in threshold in the corresponding primary sensory receiving area concomitant with an increase in threshold of other cortical areas. Other data (90) indicate that unit activity may increase, decrease, or be quite unaffected by EEG desynchronization. Hubel (49) and Evarts (31) have shown that decreased unit activity occurs in association and primary receiving areas during cortical desynchronization upon waking, suggesting a tonic inhibitory influence on many cortical neurons. Russek (99) and Purpura (87) proposed that cortical desynchronization may partially represent the activation of inhibitory interneurons.

Schadé (102) reports that slow cortical EEG potentials appear ontogenetically at the time when the dendritic plexus develops and probably arise from the apical dendrites. Unit responses can be recorded well before this time, which implies that no simple invariant relation need exist between these two levels of process.

*Orientation reflex.*—A further consequence of the presentation of a novel stimulus is the so-called orientation reflex, described by Roger *et al.* (91) as including block of the alpha rhythm together with effects on heart rate, respiration, galvanic skin response (GSR), and electromyogram, in addition to the orientative or searching movements toward the stimulus. Buser & Rougeul (18) report that a stimulus which elicits an orientation reflex abolishes the polyphasic long-latency secondary sensory responses found outside the primary projection areas.

The most widely investigated electrographic aspect of the orientation reflex has been the so-called hippocampal arousal pattern of approximately 5 cps slow waves in the hippocampus and closely related structures, accompanying neocortical desynchronization. Green & Arduini (42), who first de-

scribed this activity, reported that septal lesions prevented this characteristic response although it could be elicited in the absence of the neocortex.

Adey *et al.* (2) showed that, while septal lesions abolish the hippocampal slow wave during arousal, entorhinal lesions do not. Eidelberg *et al.* (29) have used filtering techniques to show that, during neocortical arousal, low-voltage fast activity is present in the dorsal hippocampus superimposed on the characteristic slow activity. Lesion of the septum or of the centromedian nucleus blocked the hippocampal slow wave. Large medial thalamic lesions at the dorsal medial nucleus also blocked this response to reticular or photic stimulation, whereas lesion of the anterior, lateral, or ventral posterior nuclear groups had no such effect. Grastyán (40) and Novikova & Farber (82) also described desynchronization superimposed on slow waves in dorsal hippocampus and mesencephalic reticular formation during orientation responses to unfamiliar stimuli, suggesting hippocampal desynchronization as the characteristic response to a novel stimulus.

Adey and his co-workers (2) further report that, when naive cats are first placed in a Yerkes box for pattern discrimination training, slow waves appear synchronously in the dorsal and ventral hippocampus and the entorhinal area, coupled with search and escape attempts. Grastyán (40) has observed that hippocampal stimulation blocks the orientation reflex and presents evidence (41) that rhythmic slow hippocampal activity is not an arousal pattern, but rather is related to early stages of temporary connection formation during acquisition of a conditioned response.

*Evoked potentials.*—The presentation of novel stimuli to naive, unanesthetized animals elicits widespread evoked potentials, indicating that the effects of such stimuli spread far beyond the confines of the modality-specific sensory systems. Information about the characteristics of resting activity and response to a novel auditory stimulus in layers of the auditory and motor cortex has been provided by Rabinovich (88), using a technique calculated to place one electrode in each cortical layer of these areas. Although frequency as well as amplitude changes could occur differentially in any one layer, coupling of activity frequently occurred between layers 1, 5, and 6 of the auditory cortex, suggesting a conjugate relationship between the apical dendrites and large pyramidal cell bodies in layers 5 and 6. Amplitude increases were most frequently observed in the surface layer, which showed no reflection of activity from layers 3 and 4.

A novel auditory stimulus caused all layers of the auditory cortex to show an increase in amplitude, most marked in layer 4, which also showed an increase in frequency. The slow component of the primary response was clearest in layers 1, 5, and 6. A similar increase in amplitude occurred in the motor cortex, but the response latency was longer and a frequency increase appeared uniformly in all layers. Rabinovich interpreted this to indicate extensive participation of the motor cortex in the orientation reflex.

In a study of unit responses during cortical conditioning, Morrell (76) observed that, on the initial presentation of auditory and visual stimuli, units

in the visual cortex and in the dorsal hippocampus responded only to light, units in the reticular formation responded to both kinds of stimuli, and units in the nucleus ventralis anterior responded to neither.

#### RESPONSE TO REPEATED NONREINFORCED STIMULI

*Diminution of response.*—Since the earlier studies by Hernández-Peón *et al.* (45), it has been known that repeated nonreinforced presentations of an initially arousing stimulus to an animal cause a gradual diminution and disappearance of evoked potentials and of desynchronization from the electrical record. Lessening the action of the reticular formation by lesion or by pentobarbital sodium causes "dishabituation", a reappearance of these responses. Such habituation of desynchronization or of evoked responses has been uniformly reported for a wide variety of structures in numerous recent studies, most of which were concerned primarily with electrographic changes during conditioning (8, 21, 33, 38, 43, 46, 47, 56, 58, 59, 71, 75, 82, 88, 91, 94, 103, 105, 107, 118, 119, 121). In general, these reports describe a progressive shortening of the desynchronization period or diminution of evoked response amplitude until they fail to develop, generally accompanied by increasingly widespread hypersynchronous slow activity. Workers concerned with laminar analysis (88, 94) have observed that habituation of response to a click is generalized in all layers of the cortex, but the effects of the stimulus persist longest in layer 4 of the auditory cortex. After repeated presentation of the stimulus and habituation of the orientation reflex, the afferent volley causes only nonpropagated excitation in layer 4. Unit responses in various regions (76) have similarly been observed to diminish.

In comparable fashion, repetitious stimulation results in gradual disappearance of the orientation reflex and of hippocampal slow waves. The orientation reflex vanishes before EEG habituation is complete (103), and it has been proposed that the generalized slow potentials which appear during the habituation procedure are related to the inhibitory process responsible for extinction of the orientation reflex (94).

*Specificity of habituation.*—Although the effects of habituation generalize, in that successively fewer trials are required to achieve habituation of responses to new stimuli (8), there is appreciable evidence which supports the previous conclusion of Sharpless & Jasper (104) about the specificity of the process. Roger *et al.* (91) have described the return of desynchronization, EMG, and galvanic skin responses to a visual stimulus which differs from that used during habituation. Similarly, EEG response returns on presentation of a dimmer-than-habituated light (46). John & Killam (58) observed that habituation to a flickering light occurred more rapidly in nonmodality-specific than in modality-specific structures and was frequency specific. Glickman & Feldman (37) achieved habituation of the arousal response to reiterated electrical stimulation of the brainstem reticular formation in acute and chronic cat experiments and showed that this diminution was specific to the frequency of the central stimulus.

These various data show that presentation of a novel stimulus exerts influence on widespread regions of the brain, suggesting that information about an unexpected change in the environment is distributed broadly through the diverse functional systems, where interaction with ongoing processes is potentially possible. As repeated inconsequential presentation of a stimulus establishes that it is functionally irrelevant, its influences are gradually excluded from these systems, lingering longest in the relevant sensory pathways. The specificity with which familiar and inconsequential stimuli are suppressed, while other stimuli of the same sensory modality are not, suggests that this process must in some fashion depend on the acquired stipulation of a set of input characteristics to be suppressed, rather than on gross attenuation of an input channel by a generalized tonic inhibitory process related to muscular, vasomotor, or neural effects. This process of selective exclusion of unimportant information implies the learning of a differential inhibitory response, closely related to internal inhibition as formulated by Pavlov.

*Additional afferent control data.*—The initial observation (44) that cochlear nucleus responses to clicks are suppressed as an unanesthetized cat watches a mouse has been confirmed by Gershuni (36), who reports diminution of response to click in primary neurons and auditory cortex with undiminished cochlear microphonic response, during the mouse presentation. The diminution thus does not seem to arise from changes in sound conduction. Evidence for intra- as well as intermodality suppression of response to one stimulus by another has also been reported. Horn (47) has found that the response of the visual cortex to flicker is attenuated while a cat attends to a mouse in a flickering field but not if the cat ignores the mouse, as evidenced by absence of EEG desynchronization during the interflash period. This observation suggests attenuation of neural response to "ground" while attention is focussed on "figure", in Gestalt terms. Related phenomena have been described by Hubel (48), who has found cortical units which respond to auditory stimuli only if the cat pays attention to the sound, as evidenced by an orientation response, while near-by units respond regardless of the behavioral state.

The possibility that contraction of middle-ear muscles, rather than a centrifugal inhibitory influence on peripheral neurons, is responsible for diminution of central responses to auditory stimuli during attention has been examined by several workers. Hugelin *et al.* (50) cut the stapedius muscle in the middle ear on one side, and found that after stimulation of the reticular formation the cochlear response to clicks was reduced on the intact but not the cut side. These results raise the possibility that suppression phenomena may be due to contraction of the middle-ear muscles rather than to direct neural inhibition, further supported by the report that differential conditioned contraction of the stapedius muscle can be achieved, with concomitant reduction of cochlear microphonics (108). However, diminution of response to clicks has been demonstrated at various levels of the auditory system without

comparable effects on the  $N_1$  and  $N_2$  components of the eighth nerve response (95), as has a drop in the neural but not the microphonic component of auditory response (36), under conditions which indicate the probable participation of neural inhibitory processes in these phenomena, perhaps even at the level of the first-order neuron.

The suggestion that response of the peripheral neuron can be modified by centrifugal neural influences is supported by other evidence. Reduction of the late wave of the optic tract response to light during habituation (46), influence of polarization of the occipital cortex on ERG potentials (98), and conditioning of the  $b$  wave of the ERG (10) are among the relevant findings. These data cannot be unequivocally interpreted without evaluation of the possible role of factors such as variations in pupillary diameter. During mydriasis after administration of atropine, stimulation of the reticular formation no longer causes variation in the size of photically evoked responses at the optic chiasm or in the lateral geniculate, although cortical responses still decrease (79). This suggests that RF stimulation exerts its inhibitory effect after the level of the first sensory relay. However, the direction and magnitude of the effects of RF stimulation depend on the parameters and location of both the conditioning and test stimuli (12, 69).

Although these data show that nonneural factors can contribute to variations in response to afferent input observed at peripheral levels, it seems probable that some centrifugal neural influences are directly exerted on peripheral neurons. It is clear that neural mechanisms of enhancement and suppression exist at more central levels. A number of structures seem capable of participating in this regulation (69), including the reticular formation, which is capable of exerting facilitatory or inhibitory effects, depending on stimulus parameters.

Russek (99) has analyzed the problem of the site of action of external inhibitory influences on a conditioned response. After establishment of a conditioned defensive reflex to direct stimulation of the suprasylvian cortex, a loud noise during suprasylvian stimulation blocked conditioned response (CR) performance. If the CR is established using central delivery of both the conditioned and the unconditioned stimuli, external inhibition of CR performance occurs similarly. However, movements elicited by direct stimulation of motor cortex are not blocked. These data lead Russek to reject the hypothesis that external inhibition is caused by block at the first afferent synapse. He concludes that external inhibition can interrupt transmission of impulses from the conditioned sensory cortical area to motor regions.

#### EARLY SIGNS OF CONDITIONING

*Effects of initial reinforcement.*—As a consequence of the initial pairings of conditioned and unconditioned stimuli (CS and US), one would expect that the previously habituated CS would again come to elicit behavioral arousal, EEG arousal, and the orientation reflex in the early phase of condi-

tioning. A number of studies report that, as reinforcement is added to a previously habituated stimulus, the effects of habituation are partially reversed (8, 33, 38, 56, 58, 59, 91, 105) before the initial appearance of the peripheral conditioned response. Beck and his co-workers (8) report that a gradual increase in the occurrence of arousal to the CS precedes appearance of the defensive conditioned reflex. Further relevant observations have been presented by Segundo (103) in an experiment in which the effects of direct electrical stimulation of mesencephalic reticular formation or centromedian nucleus were established as a CR by pairing with a previously habituated tone. In this situation, he noted an absence of response to the CS until 10 to 30 pairings with the central stimulation, after which the CS elicited orientation or startle responses. Subsequently, the CS evoked the characteristic response to central stimulation. Roger and co-workers (91), describing the intensification of alpha block and galvanic skin response components of the orientation reflex during CR elaboration, note that CR formation is more difficult if the orientation reflex has been thoroughly habituated.

Several investigators have observed the appearance of slow electrical activity after initial reinforcement. Maeno (105) observed 5 cps activity, shortly after the beginning of pairing of the CS and US, in several structures including the nucleus centralis lateralis, caudate nucleus, habenula, fornix, stria medullaris, and superior colliculus. Similar activity has been observed by Shumilina (107) in the reticular formation and centralis lateralis, and by Grastyán *et al.* (41) in the hippocampus. These investigators agree that neocortical desynchronization and orientation movements are elicited by the conditioned stimulus concomitantly with slow activity in the structures noted above.

An exceedingly precise and detailed analysis of the slow hippocampal activity has been reported by Adey and his co-workers (2). These authors observed a sequence of changes in the distribution and phase relationships of slow waves in the dorsal hippocampus, ventral hippocampus, and entorhinal area during pattern discrimination training in the cat. On the basis of simultaneously recorded photographic and EEG data, they suggest that 6 cps activity occurs in dorsal hippocampus and entorhinal areas during approach behavior rather than during searching head movements akin to the orientation reflex and seems to be related to the execution of a planned motor act. The 6 cps activity subsides at the completion of the motor act and appears unrelated to the accuracy or level of performance. These conclusions are relevant to the model proposed by Pribram (86), in which these structures are assigned a role in the regulation of planned sequence of actions.

Cross-correlational analysis revealed that different parts of the hippocampal arch show changes in phase angle during training in the same animal. In early training, the computed results are consistent with a pathway receiving afferent influx from septal and anterior thalamic areas, passing from the dentate granule cells in zone CA<sub>1</sub> to the hippocampal pyramidal cells in zone CA<sub>2</sub>, and thence to the entorhinal area. By contrast, in the trained

animal, activity in the dentate cells lags behind the hippocampal pyramidal cells which lag behind the entorhinal area, as if the direction of activity were reversed, with changes in relative timing from 85 to 100 ms. Slow-wave activity was maximal in the vicinity of dendrites of the hippocampal pyramidal cells. Adey suggests that the convergence of dentate axons and temporoammonic tracts on these dendrites might provide the structural basis for a phase-comparator mechanism. He raises the possibility that significant integration may occur via ephaptic transactions between dendrites without propagated cellular discharge as an inherent consequence of such processes.

#### APPEARANCE OF INITIAL CONDITIONED RESPONSES

The rather logical analysis of the conditioning process proposed by Pavlov (85) argued that the neural consequences of the conditioned stimulus

SUMMARY TABLE I  
CENTRAL NERVOUS SYSTEM PROCESSES POSTULATED IN  
EARLY STAGES OF CONDITIONING

Process	References
IRRADIATION	YES: (8, 43, 56, 71 to 73, 76, 101, 112, 113, 118) NO: (59)
CONSOLIDATION	YES: (5, 8, 43, 67, 71 to 73, 76, 101, 110, 112, 113) NO: (56, 57, 59, 91, 103, 118, 120, 121)
ASSIMILATION	YES: (56 to 58, 67, 72, 75, 110, 112, 118 to 120) NO: None

must in some fashion spread through the nervous system (irradiation) and establish a functional relationship with regions affected by the unconditioned stimulus (temporary connection) before the first signs of conditioned response can appear. Coupled, or reinforced, structures mediating the CS-US processes were postulated to retain their excitability, while those involved in nonreinforced processes would become inhibited as a consequence of an irradiating inhibition. An increasingly limited scope to the excitatory effects of the CS (consolidation) would thus replace earlier irradiation, as a CR became fully established. These propositions, which were initially formulated largely on the basis of inferences drawn from peripheral observations, can now be evaluated using current techniques which permit more direct inquiry as to the existence and nature of these processes.

*Irradiation.*—The summaries provided in Tables I and II show that extensive evidence supports the occurrence of an increasingly widespread effect of the conditioned stimulus on the central nervous system in the



early stages of conditioning, before appearance of the conditioned response. However, many of these recent electrographic studies assume that the appearance of low-voltage fast activity in a structure is a manifestation of diffuse excitation. Kogan (62) reports the results of using motor cortex stimulation as the US to provoke movement subsequently established as a CR to an external stimulus. He investigated changes in the threshold of this and adjacent motor points as the CR became established. Early in conditioning, the CS began to evoke increasingly marked desynchronization in the cortical sensory area, accompanied by generally decreased thresholds for elicitation of movement by direct cortical stimulation (irradiation). As conditioning proceeded, this motor excitability increased further, particularly at the reinforcement point, and then, as the conditioned response became established, CS presentation caused a decrease in threshold only at the reinforcement point, while causing an increase in threshold in surrounding regions of the cortex (consolidation). However, desynchronization appeared over broad regions of cortex during the CS, outside as well as inside the zone of excitation in which thresholds were lowered. This, and other evidence of excitability decrease as well as increase accompanying desynchrony, places in doubt some of the interpretations of extensive desynchronization during CR formation as sufficient evidence for the irradiation of excitation.

Doty & Giurgea (27) have described a decrease in the current necessary for central elicitation of a conditioned response as training proceeds, suggesting that the region into which this "central CS" propagates has an increased excitability. Doty & Rutledge (25) also suggest an increase in excitability of the motor system of the conditioned limb, basing this suggestion on behavioral observation.

Kogan describes, in addition, the development of hypersynchrony and slow waves during differential conditioning and relates this to a decrease in excitability. While external inhibition, as from presentation of a sensory stimulus, is characterized by desynchrony, internal inhibition, as developed differentiation or extinction of a conditioned response, is characterized by hypersynchrony. Both of these processes, interestingly, will spread to undercut motor cortex, suggesting partial mediation by some transcortical process.

Grastyán (41) proposes that rhythmic slow activity indicates the development of temporary connections in which the hippocampus participates. He argues that the development of hippocampal slow waves early in conditioned response formation indicates involvement of the reticular formation and hypothalamus in initial phases of connection formation, since their activation can evoke hippocampal slow waves. He proposes further that the later desynchronization of the hippocampus is a sign of increased excitation responsible for inhibition of the orientation reflex as the CR stabilizes. Yoshii *et al.* (119) propose a related hypothesis, accounting for the generalized desynchronization in the first stage of cortical conditioning as indicative of mesencephalic RF activity, and the conditioned suppression of brain-

stem RF activity in the later stages as responsible for the diminution of desynchrony and its replacement by frequency-specific repetitive response.

John & Killam (56) have described a detailed set of changes in the distribution of frequency-specific responses to a flicker CS during conditioned avoidance response elaboration, including an increased response to the CS early in training in the reticular formation and the hippocampus, and replacement of this response later in training by a response confined to the nucleus ventralis anterior and visual system structures. In a subsequent report, John & Killam (58) have studied changes in the distribution of frequency-specific responses to a flicker CS during simple approach conditioning, differential approach conditioning, and differential approach-avoidance conditioning in the cat, describing a sequence of changes different from that observed in avoidance conditioning. Some aspects of the earlier report have subsequently been confirmed by Liberson and his co-workers (67), but the latter workers observed a terminal disappearance of electrical response as the conditioned behavior stabilized. Morrell (76), studying the response of units located in the visual cortex, reticular formation, dorsal hippocampus, and nucleus ventralis anterior during cortical conditioning, observed that, as the conditioning procedure was initiated, after suppression of unit response everywhere by habituation, increased unit responses to the CS appeared in the visual cortex, reticular formation, and dorsal hippocampus, together with general cortical desynchronization; the appearance of frequency-specific repetitive response to the CS in the visual cortex was correlated with suppression of visual units to the CS, which decreased the activity of reticular formation units while increasing the activity of dorsal hippocampal units; the appearance of localized desynchronization to the CS, confined to the visual cortex, was accompanied by increased unit activity in the visual cortex, absence of response of reticular formation and dorsal hippocampal units, and increased unit response in the nucleus ventralis anterior units. It is of interest that this sequence parallels that reported by John & Killam (56) during avoidance training. Morrell suggests that these results support the proposals advanced by Gastaut (34) and Yoshii *et al.* (118) that participation of elements in the thalamic reticular system is essential for final development of a CR and that a fully established CR involves some form of link between this system and cortex. It may be recalled that Gastaut based his hypothesis on the observation that localized desynchronization, such as is obtained by thalamic but not by midbrain reticular formation stimulation, characterized the electrographic expression of closure in the well-established conditioned response. This formulation is difficult to reconcile with the report by Chow *et al.* (20) that destruction of the rostral thalamus is compatible with performance of complex pattern discrimination responses. However, the persistence of some recruiting responses in these lesioned animals may indicate some alternative corticopetal pathways from the thalamic diffuse projection system other than the final common pathway suggested through the rostral thalamus.

Rabinovich (88) has described changes in the various layers of the auditory and motor cortex during establishment of a conditioned defensive reflex to an auditory conditioned stimulus. After initial pairings of shock with the CS, an increase in amplitude lasting for the duration of the CS and a shorter frequency increase lasting several seconds were observed in all cortical layers, appearing after the slow component of the initial response. With continued pairing of shock with the CS, as the conditioned response became elaborated, these changes in activity became briefer, and were most marked in layer 4 of the auditory and layer 5 of the motor cortex before onset of the motor response. Following this heightened activity, a well-synchronized slow (2 to 3 cps) potential at all levels indicated a decrease in activity as the response proceeded. On occasion, slow waves were observed which were isolated to layer 2 of the motor cortex, and not recorded from the surface. Presentation of the CS during such slow waves usually failed to elicit the CR, even in well-trained animals, although response was observed in the auditory cortex.

On the basis of such data, Rabinovich proposes that afferent input to the auditory cortex causes outflow from layers 3 and 4 via association fibers to pyramidal neurons, ultimately reaching the efferent link by way of the stellate cells in layer 2 of the motor cortex, from which short axons excite the pyramidal cells in layer 5 causing the outflow into the pyramidal tract of the discharge producing the motor response. A similar process is proposed by Roytbak (94) on the basis of somewhat more inferential reasoning.

Morrell (76) has conducted laminar analyses of the phenomena of cortical conditioning. After systematic combination of steady tone with flickering light, he observed a clear response to the tone alone occurring at the frequency of the flicker. This frequency-specific repetitive response to the steady tone was obtained from surface visual leads but not from leads 2.0 mm. below the surface. In the later stage, a localized surface desynchronization occurred coinciding with a rapid deep discharge to tone. Morrell interprets these data as indicative that the conditioned linkage is formed along the synaptic membrane of the apical dendrites, and suggests that the coding of information may be achieved by the patterning of hyperpolarizing and depolarizing postsynaptic potentials elicited in the apical dendrites by the influence of interneurons and inputs from unspecific afferent systems.

*Temporary connection formation.*—A number of experiments have been reported which are directed towards elucidation of the connection formation process itself. Some years ago, Rusinov (98) reported on the effects of establishment of a so-called "dominant focus" by surface anodal polarization of a region of motor cortex. Repeated presentation of a sensory stimulus initially without motor effects, during continuous maintenance of this low anodal current (2 to 10  $\mu$ A), ultimately came to elicit motor movements corresponding to the polarized region. On cessation of the polarizing current, presentation of the sensory stimulus continued to elicit the motor response

for periods up to half an hour, and this effect gradually diminished and disappeared. Data confirming these observations have been obtained by Morrell (78) who also noted that presentation of previously ineffective tones or flickering light during unilateral anodal polarization of the motor cortex elicits limb movements. In addition, Morrell noted that presentation of the peripheral stimulus during motor cortex polarization evoked a change of motor area unit discharge frequency, although, previous to polarization, the peripheral stimulus did not alter the rate of unit firing. However, these evoked changes in motor unit activity are related neither to the presence nor the absence of limb movements elicited by the peripheral stimulus. When flickering light was used, assimilation of the rhythm was observed in motor cortex records. This phenomenon is manifested primarily when the peripheral stimulus fails to produce the usual limb movement. These findings are reminiscent of similar observations in experiments involving orthodox conditioning methods (72, 90).

Morrell also determined the effects of cortical polarization on incompletely elaborated conditioned avoidance responses when a photic stimulus was used as the conditioned response. In this experiment, polarization of the motor cortex did not affect performance. However, anodal polarization of the visual cortex resulted in a marked suppression of the conditioned response.

Rusinov (96) has further reported that, if a dominant focus is maintained first in the forepaw region until a previously indifferent stimulus elicits forepaw flexion, and the polarizing electrode is subsequently shifted to the hindpaw region, then presentation of the previous stimulus elicits both forepaw and hindpaw flexion. If stimuli are repeated with too short an intertrial interval, an inhibitory effect occurs which can be reversed by increasing the interval. Along similar lines, Rusinov describes experiments of Pavlygina (97) in which the orientation response to visual and auditory stimuli is extinguished. After it is established that these stimuli do not alter respiration or heart rate, they are presented repeatedly during maintained hypothalamic anodal polarization. Both light and sound reduce heart rate and increase respiration, i.e., the previously indifferent stimuli persist for some hours or even days after termination of the polarization. With cathodal polarization, no effects are observed. If conditioned responses are established in orthodox fashion to a CS before hypothalamic polarization, these conditioned responses are reduced or blocked during such polarization, and the CS elicits the hypothalamic response rather than the previous conditioned response. This seems to suggest that the dominant focus in the hypothalamus in some fashion reroutes the CS and pre-empts the response thereby elicited. Perhaps a mechanism of comparable sort, which normally helps to mediate the CR, is successfully overridden by the applied polarization. Rabinovich & Trofimov (89) have reported that a cortical dominant focus arises during defensive CR formation.

Chocholová (19) has attempted to apply these findings to explain the

conditioned diminution of audiogenic seizure in rats. She used an "epileptogenic" sound stimulus (of briefer duration than the latent period for production of audiogenic seizures) as the CS for an alimentary conditioned response. After establishment of the conditioned response, prolongation of the sound beyond the period previously effective in provoking seizure caused few or no seizures. This effect was attributed to the rerouting of the excitatory input to a new dominant focus mediating the path of conditioned alimentary response, and thus diverting it from the epileptogenic region.

Podsosnaia (97) established a dominant focus by gradually diminishing the intensity of a 12 per minute electrical shock to the foot. Indifferent stimuli to which the orienting reflex had previously been extinguished now caused a rhythmic 12 per minute defensive flexion of the foot, although these sensory stimuli were steady. This seems to indicate that the dominant focus retains characteristics related to the temporal pattern of the stimulus which established it, and raises the question of possible relationships between this phenomenon and the rhythmic response elicited by a tone previously paired with flicker in the cortical conditioning paradigm.

Roytbak (93) described a procedure which establishes an analogue to the dominant focus by intense neural stimulation. He shows that slow large hypersynchrony of irregular frequency arises in a cortical region after cessation of a tetanizing stimulation. Stimulation of a distant cortical point at frequency F, which normally cannot be demonstrated to alter electrical activity in the first region, causes discharge at frequency F in that region if it occurs immediately after cessation of the tetanizing stimulation.

Yoshii *et al.* (117) cite a study by Naumova in which the motor cortex was polarized, and repeated auditory stimulation came to evoke limb movements. When hypersynchronous waves appeared in the EEG of the motor cortex, the auditory stimulus failed to evoke movements, in spite of the continued anodal polarization. Sokolova (109) has provided EEG data of visual and motor cortex responses to visual and auditory stimuli which elicited motor movements during anodal polarization of the motor cortex. She reports that when a motor response is obtained to a sound, the sound elicits first a suppression of activity in the visual cortex, then slow waves followed by a desynchronization of motor cortex as the movement occurs. When steady light is used as the stimulus, as "closure" develops and the movement appears, the major EEG change seems to be a marked increase in the similarity of visual and motor cortical electrical activity in the intertrial interval, suggesting that a functional coupling has been established between these regions.

The progression of events seems somewhat clearer when flickering instead of steady light is used as the peripheral stimulus. On initial presentation of the flicker, clear frequency-specific responses appear in records of electrical activity of the visual cortex and are much less apparent or absent in motor derivations of either side. As anodal polarization was applied to the right motor cortex and flicker presented repeatedly, frequency-specific

potentials appeared clearly in right but not in left motor cortex, and left foreleg movement appeared. During the intertrial period, the right motor cortex showed an increase in frequency and amplitude with a high degree of similarity to the right visual cortex. These similarities between regions correspond to some reports of electrographic similarities observed between regions during the phase of "generalization" during elaboration of a defensive conditioned response to flicker, and raise questions as to the possible identity of the mediating processes. Preliminary observations in our own laboratory (65) in acute curarized preparations have revealed electrographic changes of this general sort with a very similar procedure. The established frequency-specific responses to flicker in polarized motor regions persisted for long periods after polarization was terminated and were abolished by undercutting the motor cortex.

The mechanism mediating phenomena of this sort is not clear, but a number of previous findings may be relevant. Burns (17) has reported that surface-positive polarization of isolated cortical slabs was excitatory, while anodal micropolarization 1.3 mm. below the surface was ineffective. Cathodal polarization had the converse effect. Babsky *et al.* (6, 7) showed an increase in cholinesterase activity and a decrease in acetylcholine activity in anodally polarized regions of the cat cortex and the converse cathodal effect, compared to unpolarized regions. They proposed that the responsible mechanism was activation of cholinesterase by accumulation of bivalent ions at the anode with a consequent diminution of acetylcholine due to increased hydrolysis and a converse effect of monovalent ion accumulation at the cathode. This suggests that the effects of an anodal dominant focus might be mimicked by a sustained small increase in surface calcium ion.

In view of the apparently facilitatory effect of small DC currents on the establishment of new functional relations in these experiments, the report by Morrell (76) that conditioned localized slow potential shifts can be established suggests that this mechanism may be of functional significance in the establishment of conditioned responses of more orthodox form. Low-intensity tone, which of itself did not produce a DC shift, was paired with stimulation of the centromedian nucleus (CM), which resulted in a 500  $\mu$ v. to 1 mv. negative DC shift on the central cortex ipsilateral to the side of stimulation, with a pronounced and long-lasting surface-positive shift occurring at the end of central stimulation. After about 30 paired trials, tone alone caused a similar DC shift, localized to the side of previous centromedian nucleus stimulation. These DC shifts were often but not always associated with cortical desynchronization. It has previously been reported that DC shifts of this sort arise from summation of after-potentials primarily in apical dendrites of the surface layer, which supports Morrell's proposal that at the cortical level temporary connection or closure occurs in the upper layer, i.e., in dendrites.

These various phenomena suggest the possible role in the mediation of electrical aspects of early temporary connection or closure of a process which, for want of a better term, we might describe as analogous to "con-



ditioned posttetanic potentiation". It seems highly desirable to obtain data hitherto not available about the parametric aspects of these phenomena, in particular whether they can be established differentially and how the duration of aftereffects varies with the number of paired trials, to evaluate whether this is a process of localized sensitization (of itself potentially significant) or one with more differential characteristics.

Although short-term electrotonic or reverberatory maintenance of the temporary connection may be contemplated with equanimity, long-term mechanisms of memory seem to necessitate a structural modification of some sort to achieve sustained changes in excitability to specific inputs. It seems highly unlikely that inhomogeneities in distribution of highly labile ions could account for long-lasting alterations of membrane potentials. Modifications in the spatial distribution or concentration of less labile substances seem a necessary postulate for permanent mechanisms of excitability alteration. Yet, isotope studies reveal that substances in the brain have high rates of turnover—synthesis, breakdown, and resynthesis. Therefore, one must postulate the involvement of a substance which can reproduce or perpetuate a pattern of molecular components. The search for such a substance leads logically to the nucleic acids, and the requirements of cytoplasmic locus for that substance, to ribonucleic acid.

Morrell (77) has reported experimental observations based on this line of reasoning, with highly promising results. If a chronic epileptogenic lesion is produced by local application of ethyl chloride spray to a 2 mm. square area of cortex, in several days epileptiform spikes are observed not only in the region of the lesion but in the symmetrically located contralateral region (mirror focus). This secondary activity is dependent at this stage, in that it subsides after ablation or isolation of the primary lesion. In the cat, after a period of several weeks, the secondary contralateral focus becomes independent, in that it does not disappear upon ablation or coagulation of the primary discharging focus. Once this independent stage has been reached, local increases in neuronal excitability may be demonstrated electrically, and suggest that the secondary focus is a network in which spontaneous and evoked activity have been chronically altered as a consequence of a sustained alteration of afferent input. Neuronal isolation of the secondary focus, after independence has been achieved, results in replacement of the increased electrical activity by electrical silence. After such isolation, several months were permitted to elapse, during which surface recordings from above the isolated cortical slab indicated no return of paroxysmal activity. At the end of this period, an acute experiment was performed during which pentylenetetrazol-induced seizures initiated on normal cortex adjacent to the isolated slab were demonstrated to spread onto the slab. Such ephaptic transmission of seizure does not occur in isolated but nonepileptic cortex, and is adduced as evidence that increased excitability has persisted in the mirror focus region. Morrell proposes that this demonstrates that the mirror focus is a region which has not only "learned" the paroxysmal discharge but has retained an increased excitability even after several months of inac-



tivity, thus excluding reverberating impulses as the basis for maintaining the changed electrical activity and indicating the probability of local structural alterations.

The ribonucleic acid (RNA) distribution in this neural "learning" model was investigated and compared with that of electrically uninvolved adjacent regions, by use of methyl green pyronin staining. High-power photomicrographs show that the pyronin-positive material in the region of the mirror focus (not present if sections were pretreated with ribonuclease) was distributed densely in the dendrites in a layer along the inner surface of the cell membrane and is not observed in this fashion in the normal cortex, nor in the cortex contralateral to a simple cortical excision. Morrell suggests, therefore, that the altered RNA distribution is the chemical correlate of the structural modification and that, since the mirror focus has some of the attributes of learning and of memory, perhaps the complex of ribonucleic acid and protein is an essential element in the molecular basis of memory.

The excellent and provocative work of Morrell should be somewhat extended to rule out factors as yet uncontrolled, in view of the potential significance of this work. Echlin (28) has reported that the chronically isolated cortical slab is hypersensitive to acetylcholine, although no mirror focus characteristics have been attributed to it. Hydén (52) has shown that RNA synthesis is increased markedly by brief neuronal stimulation. One wishes that control data, undoubtedly forthcoming soon, were already available on RNA distribution in the nonepileptic chronically isolated and subsequently stimulated slab and in postparoxysmal normal cortex. These data would contrast with the picture already provided of the chronically isolated slab after demonstration that an excitability increase has been sustained.

Hydén and his co-workers (11, 52) have previously demonstrated increases in cellular ribonucleic acid with various kinds of simulation. Hydén (52) has integrated these observations to propose a theoretical model for the intracellular mechanism of memory which is based on altered ribonucleic acid-amino acid relationships as a consequence of ionic flux. Kreps (64) has presented evidence for altered RNA synthesis in regions of the nervous system related to the conditioned stimulus after establishment of a conditioned response.

In a number of experiments based on the reasoning outlined above, we have observed effects which may implicate RNA in the mechanism of retention of experience. Intraventricular injection of ribonuclease into conditioned cats has repeatedly been followed by a temporary deterioration of pattern discrimination, while avoidance CR performance was unaffected (John, Wenzel, and Tschirgi, unpublished data). More recently, we have studied the effects of ribonuclease on retention of a defensive CR by regenerating planaria. Previously conditioned animals were transected and permitted to regenerate in pond water containing ribonuclease. Animals regenerated from head sections showed equal retention of CR whether

regeneration occurred in the presence of ribonuclease or not. While animals regenerated from tail sections in the absence of ribonuclease showed the same amount of retention as did head sections, animals regenerated from tail sections in the presence of ribonuclease showed no CR retention, although they can subsequently be retrained (Corning and John, unpublished data). Although desirable histochemical and histological data have not yet been obtained, these data plus those previously mentioned constitute a promising line of investigation warranting further exploration.

#### CHARACTERISTICS OF THE WELL-ESTABLISHED CONDITIONED RESPONSE

The essential element in the Pavlovian formulation of the conditioning process is the establishment of a temporary connection between neural regions subserving the conditioned stimulus and those subserving the unconditioned stimulus. As a consequence of this new functional relationship, the effects of the CS are rerouted to the US region, exciting those structures previously activated only by the US, thus eliciting a conditioned response. In the previous section we discussed recent data relevant to possible mechanisms of connection formation. Three salient questions arise about the processes mediating performance of the fully established conditioned response. Does the CS gain access to the US region and by what pathways—where does closure of the temporary connection occur? What characteristics does the temporary connection possess? Does the postulated spreading inhibitory process cause a diminution of the effects of the conditioned stimulus in noncoupled regions after closure is achieved—what is the evidence for consolidation?

*Pathways of closure.*—A number of data oppose the assertion that the conditioned stimulus is routed to the US region following conditioning. After using electrical stimulation of the reticular formation as the US for establishment of a CR to tone, Segundo *et al.* (103) showed that coagulation of the region to which the US had been delivered did not abolish the ability of the CS to elicit conditioned responses characteristic of the effects of stimulation. This indicates that the CS effect could not be attributable to activation of the US region itself. Describing the results of microelectrode studies of unit activity in various cortical regions during avoidance conditioning, Jasper and his co-workers (53, 90) report that motor cortex units showed grouped firing at the flicker CS frequency only if the CR did not appear or during extinction, at which time the surface EEG showed frequency-specific response. [Similar EEG observations have been made by others (72, 114).] These workers concluded that it was necessary to look elsewhere than in the motor cortex for convergence of impulses critical to the activation of neural pathways of the conditioned response. In contrast, groups of unit responses with the temporal pattern of the flicker CS appear in parietal cortex during avoidance conditioning, raising the possibility of convergence there with secondary evoked responses to the unconditioned stimulus. It is particularly interesting that when erroneous CR's were per-

formed to the differential stimulus during differential conditioning, these units showed their usual activation pattern, although normally they were suppressed during the differential conditioned stimulus.

Observing consistent diminution in the amplitude of occipital evoked potentials to the CS just preceding the CR, Jasper *et al.* suggested that either the cortical receiving area or some other part of the afferent pathway of the CS might participate in CR mediation. The results of Doty & Rutledge (25) suggest that the temporary connections established during CR formation are at least one synapse removed from the cortical system of the conditioned stimulus. These workers established flexion CR's using electrical stimulation of various cortical regions as the CS. The ease with which a CR initially established to stimulation of a cortical sensory area as CS could be transferred to a peripheral CS of the corresponding sensory modality shows that the mechanisms which mediate CR's to a centrally delivered CS are validly comparable to those mediating CR's established in more orthodox fashion. Marked ease of transfer of CR was found from cortical to peripheral, peripheral to cortical, cortical to homotopic cortical, and cortical to heterotopic cortical stimulation as the conditioned stimulus. Large cortical extirpations which included the point of application of the central CS, after establishment of the CR but before test of transfer to homotopic cortex, did not interfere with transfer, showing that the CS region itself was not essential for CR performance.

Corticofugal rather than transcortical pathways mediate the CR, since circumsection of the site of central stimulation did not prevent retention, whereas undercutting the stimulated cortical region abolished the CR (24, 25). Transcallosal connections are not essential since complete generalization can be observed between homotopic points even if callosal section is performed before initial conditioning begins. These conclusions are supported by the work of Doty & Giurgea (27), which shows that, after complete callosal section, a CR could be established by delivering a CS to one hemisphere and a US to the other. Success was also achieved in the establishment of differential CR's between stimuli delivered to homotopic points on the two hemispheres. Rutledge & Kennedy (100) have found a delayed wave complex following the transcallosal response, which survived callosal section. This delayed wave showed habituation to a specific stimulus and was susceptible to external inhibition by a peripheral stimulus. The multisynaptic diffuse system presumed to mediate this response may perhaps play a role in the mediation of transfer of CR's after callosal section.

Participation of the specific thalamic relay in performance of CR's to a central CS seems unlikely in view of the report that cats trained to respond to stimulation of the medial ectosylvian gyrus perform perfectly, with no alteration of threshold, after callosal section plus destruction of the medial geniculate body ipsilateral to the central conditioned stimulus (24).

Zuckermann (122) has used the effects of local or generalized seizures on CR performance to analyze the participation of various regions in closure. Peripheral CS evoked CR performance immediately after termina-

tion of generalized cortical seizure, while the cortex was almost isopotential and also during the period of postparoxysmal slow waves, but the CR subsequently disappeared for an extended period although fast low-voltage activity with no paroxysmal discharge was observed in the cortex. In contrast, after generalized seizures caused by stimulation of the brainstem reticular formation, CR's were briefly absent and then reappeared, to be performed with unusual vigor for a prolonged period while intense cortical desynchronization persisted. Since unconditioned reflexes involving brainstem centers (e.g., the righting reflex) are inhibited for long periods after such seizures, although CR performance is intact, Zuckermann proposes that closure must occur in synapses rostral to the mesencephalon.

While Zuckermann's data seem to exclude the brainstem reticular formation as the site of closure, this structure can exert influence on neurons elsewhere which are more intimately involved. Facilitatory effects of weak RF stimulation were found on the elaboration of new CR's, and on establishment of differentiation, although such stimulation did not disinhibit a previously extinguished CR. Further, he demonstrated that RF stimulation restored performance of a CR blocked by experimental stress, a finding similar to that reported by Endrőczy *et al.* (30).

Convulsive discharge relatively restricted to the cortical region of the US exerted no postseizure inhibitory effect on the CR, although the CS failed to elicit evoked potentials on specific sensory cortex (122). This finding strongly suggests that the US region itself is not involved in mediation of the CR nor is the CS region from which evoked response is absent. However, localized seizures initiated by direct stimulation of the CS area block CR performance for an extended period following termination of seizure discharge, suggesting that functional integrity of the corticofugal projections from the CS but not the US region is a prerequisite for CR performance.

These conclusions are supported by the observations of Morrell (78) that, although bilateral anodal polarization of motor cortex with weak current had no effect on performance of a flexion CR to a photic CS, similar polarization of visual cortex resulted in a prolonged suppression of conditioned response. These findings may be related to phenomena described by Bureš *et al.* (14 to 16) who have studied the effects of potassium-chloride-induced spreading cortical depression (SD) on performance of previously elaborated conditioned responses. During the period of this cortical depression, characterized by a brief negative DC shift followed by a sustained positive shift as well as depression of primary cortical responses, avoidance and alimentary CR's were inhibited. An increase in the rate of firing of mesencephalic reticular formation units paralleled the duration of spreading depression. Some data have appeared (56, 76, 120) directly showing diminution in RF activity as CR elaboration was completed, and a number of theoretical formulations (34, 41, 105, 118) have inferred a diminution of mesencephalic RF activity as a CR becomes elaborated. Were this so, postictal depression of RF would not be expected to interfere with performance of a previously established CR, whereas increased RF activity resulting from interference with corticoreticu-

lar projections by local discharge (122), anodal polarization (78), or cortical spreading depression (16) might be expected to cause deterioration of performance.

A rather primitive CR can be elaborated during cortical SD in one hemisphere (16). When animals that have achieved criterion performance during unilateral SD are tested with SD in the contralateral hemisphere, no retention of the CR is evident. If SD is subsequently induced in the original hemisphere, CR is unequivocally present. Bureš suggests that this demonstrates that the learning involves the intact hemisphere exclusively. In view of Bureš' own demonstration that the firing rate of mesencephalic RF units is increased in RF regions contralateral but not ipsilateral to the depressed hemisphere, subcortical structures may well be implicated.

These workers (15) have also reported that topical application of penothal or thermocoagulation of the cortical sensory area did not interfere with CR performance. While these results are compatible with other findings discussed earlier, it is difficult to interpret the further observation that the localization of spreading depression to various cortical areas by protection with topical bivalent cations shows no regional differences in the effects of such depression on the conditioned response.

Using an ingenious technique which seems to achieve conditioning of single-unit responses, Olds & Olds (83) found that neocortical units never showed rapid response modification, while units in hypothalamic and rhinencephalic regions showed extensive modifiability of response. They have described also (83, 84) the use of central stimulation to achieve disruption of acquisition or retention of a daily response pattern of behavior in the rat. No disrupting effects of neocortical, reticular formation, or sensory thalamic stimulation were observed. However, hippocampal stimulation seemed to cause confusion and failure to establish appropriate response, although it did not abolish a previously stabilized response. This effect was not related to a competing reinforcing action of the central stimulus. These workers propose the hypothesis that instrumental conditioned reflex establishment may crucially depend on alteration of firing patterns in the hypothalamic-paleocortical system.

The conclusion that perturbation of hippocampal function in the rat interferes with CR acquisition is supported by lesion data provided by Kimura (61). Investigating the effects of hippocampal SD on CR performance in the rat, Bureš (16) has noted that, if SD is induced immediately after a training period, no retention of CR is demonstrable in a subsequent non-SD test period. In contrast with the absence of effect of hippocampal stimulation on retention, previously elaborated CR's are inhibited during the period of spreading depression. Rather opposite results have been reported for the cat, where Grastyán (40) and Hunt & Diamond (51) found no effect of bilateral hippocampal resections on CR acquisition, and Flynn (32) showed that the presentation of conditioning trials during afterdischarges after hippocampal seizure did not prevent learning. Hunt & Diamond, further, have described

severe impairment of a previously elaborated CR to an auditory but not to a visual CS following hippocampal lesion.

The capacity of the hippocampus to participate in the mediation of temporary connection seems indicated by the findings of Liberson *et al.* (67) that by pairing 3 cps flicker and 3 cps unilateral hippocampal stimulation several times, a food CR which was previously elaborated to the flicker CS could subsequently be elicited by 3 cps hippocampal stimulation alone. Although initial stimulation of the contralateral hippocampus at 3 cps was ineffective, brief paired stimulation of the two hippocampi resulted in subsequent performance of the CR on 3-cps stimulation of the contralateral structure alone.

A number of findings have led to the suggestion (3) that the pathway of closure must run through the hypothalamic center of the unconditioned reflex. Wyrwicka *et al.* (115) showed that in a satiated animal a food CR could be elaborated only during simultaneous stimulation of the hypothalamic feeding center. Further work (3, 114) showed that previously elaborated feeding and drinking CR's could be elicited, even in satiated animals, by stimulation of the feeding and drinking centers in the hypothalamus. Wyrwicka (116) reported that cessation of hypothalamic stimulation could maintain a previously elaborated CR, suggesting that inhibition of feeding center activity resulting from food may stabilize the conditioned response. The implication of hypothalamic participation in closure seems buttressed by the report of Olds (83) that after establishment of a food CR to a flicker CS, the CS evokes potentials at the flicker frequency in hypothalamic regions where the self-stimulation rate covaries with hunger, although previous to conditioning such potentials were not observed. These findings recall the earlier reports by Grastyán and his co-workers (39, 68) that stimulation of appropriate hypothalamic centers could elicit performance of a previously elaborated food CR simultaneously with inhibition of an avoidance CR, or vice versa. A similar reciprocity was noted for regions in the mesencephalic reticular formation.

*Characteristics of the temporary connection.*—A salient aspect of the temporary connection which has emerged from electrographic studies of differential familiarization, cortical conditioning, cyclic conditioning, classical and instrumental conditioning, approach and avoidance conditioning, generalization tests, and trace conditioning is the ability of the system established by repeated experience with an intermittent stimulus to represent the temporal characteristics of that stimulus. The data for such "assimilation" are consistent, as can be seen from Table I, and have recently been briefly summarized (57).

Using an electronic frequency analyzer and a trace conditioning procedure, Stern *et al.* (110) confirmed the persistence of frequency-specific potentials during the delay period after an intermittent conditioned stimulus. The finding of John & Killam (56) that various structures displayed frequencies related to the initial CS during generalization of an avoidance CR to a new CS of a different frequency has received support from other re-

searches showing the persistence of previous response characteristics after changes in the stimulus (41, 72, 75, 105, 112, 118, 122). Liberson (67) has described the persistence of hippocampal waveforms characteristic of response to a particular stimulus frequency for some time after change of frequency, a finding which may be related. The possible functional relevance of assimilation is indicated by evidence that peripheral motor response can appear at subharmonics of the frequency of an intermittent CS (72, 122), by the marked ease of transfer of CR between conditioned stimuli of the same frequency but different sensory modality (56) or site of central application (67), and by the report of Neff *et al.* (80) that generalization occurred immediately to clicks at the same frequency as central stimulation of auditory paths used for elaboration of an avoidance CR, and vice versa.

*Evidence for consolidation.*—It can be seen from Table I that the electrographic evidence for consolidation of the excitatory effects of the conditioned stimulus to the cortical region of the unconditioned stimulus is far from unanimous. A number of studies have unequivocally demonstrated diminution of response with CR stabilization. Such data have, for the most part, come from studies of the distribution of evoked responses to the CS after establishment of defensive or avoidance conditioned responses. Evidence against consolidation has come, primarily, from studies using the distribution of desynchronization as the index for the extent of CS effects. Although such desynchrony is usually interpreted as indicating excitation, the work of Kogan (62) showed this to be a gratuitous assumption, as did a number of other studies cited earlier. Zuckermann (122) has commented on the absence of a constant relation between the state of cortical synchrony and CR activity, suggesting that the presence of a synchronous or asynchronous background reflects processes relatively independent of the conditioned response. Thus, evidence about consolidation based on persistence of desynchronization to the CS cannot be interpreted without ambiguity.

Consideration of the studies of John & Killam (56, 58), showing changes in the distribution of frequency-specific EEG responses to a flicker CS during conditioning, which provide that portion of the evidence against consolidation not based on the distribution of desynchronization, may assist in clarification of some discrepancies in evidence relevant to consolidation. These workers observed differences between aspects of the electrographic patterns occurring during approach and avoidance training. For example, although some evidence for consolidation after establishment of an avoidance CR appeared in the form of diminished responses to the flicker CS in the mesencephalic reticular formation and dorsal hippocampus, no such diminution was noted during approach conditioning for food; 40 cps bursts (66) were less prominent in the amygdala in approach than in avoidance CR performance, and multiple frequency response to the flicker CS characterized the response of the visual cortex in avoidance but not approach CR training.

It seems possible that the urgency of the avoidance CR results in a greater automatization of behavioral response to the CS, a broader generalization gradient of response with lesser discrimination of the specific stimulus char-



acteristics. The possibility of somewhat different arousal systems mediating alimentary and defensive CR's, raised earlier by the work of Grastyán *et al.* (39, 68), must be contemplated. Anokhin (4) has presented evidence which he interprets as indicating the existence of two arousal systems in the reticular formation, differentially related to mediation of conditioned defensive and alimentary reflexes. In the so-called defensive dominant state, an animal is characterized by a sustained cortical desynchronization and no alimentary response to a CS, although a defensive CR is often erroneously elicited. Chlorpromazine abolishes the defensive dominant state, as evidenced by return of the alimentary conditioned response. The alimentary stimulus now causes cortical desynchronization while the defensive CS no longer elicits either the defensive reflex or cortical desynchrony. Gavlichek (35) observed similar phenomena and likewise suggested separate reticular formation mechanisms for defensive and alimentary activation. Evidence provided by John *et al.* that reserpine (54) or intraventricular ionic injections (55) interfered more with avoidance CR's than with approach CR's to visual CS's seems compatible with these formulations. Thus, some portion of the discrepancy in the consolidation data may be ascribable to differences in drive dimension in the particular CR procedures used.

Killam and co-workers (60) have presented data indicating a phase of chlorpromazine action in which well-established CR's are blocked and all signs of afferent input are absent from the EEG. They also report that during the appearance of reserpine block of avoidance CR's, the EEG changes observed during acquisition are essentially reversed, except that the hippocampal responses to the CS seen early in training do not reappear. As the reserpine effects wear off and CR returns, the EEG changes seen previously during initial acquisition are recapitulated. These workers conclude that reserpine does not block afferent input but interferes with the coupling or temporary connection between input and output mechanisms which themselves remain intact. Perhaps most important, they provide data suggesting that the neurophysiological effects observed after administration of a drug may depend on the previous experience of the animal, raising the possibility that full understanding of mechanisms of action of centrally active substances may require knowledge available only from conditioned animals.

#### DIFFERENTIATED CONDITIONED RESPONSES

Another consideration seems relevant to the problem of consolidation. If a conditioned response is established to a conditioned stimulus under contingencies such that appropriate response can be performed based simply on perception of the presence or absence of stimulation in a particular sensory modality (existential discrimination), diminution of the electrical signs of response to that stimulus might be expected. Behaviorally, a broad generalization gradient should result with reliable performance of the CR to many sensory stimuli differing from the original conditioned stimulus. Were the reinforcement contingencies altered, so that a specific attribute of the CS, for example the frequency of a flickering light, rather than merely its presence

or absence, became the relevant discriminandum (differential discrimination), a sharp peak might be expected to appear in the generalization gradient, accompanied by a marked increase in electrographic response related to the stimulus frequency.

Majkowski (72), having obtained clear evidence of consolidation, as a CR was fully elaborated to a flickering light, presented a differential nonreinforced stimulus—a light flickering at another frequency. He observed a resurgence of electrographic response in structures where such response had lapsed. It is particularly interesting that while in the visual cortex the evoked responses were at the frequency of the new stimulus, in the motor cortex they were at the same frequency as the stimulus used to establish the conditioned response initially. Thus, the differential stimulus, somewhere in the course of movement from the CS region to the US region, activated a temporary connection which acted upon the motor but not the visual cortex in a fashion reflecting the temporal pattern of the CS which initially established closure.

John & Killam (58), studying differential approach-avoidance conditioning to two flicker CS's differing in frequency, observed an increase in the regularity and extent of frequency-specific responses to the CS as the attribute of frequency was made progressively more crucial. Further, when an avoidance CR was elaborated differentially after previous approach conditioning, no diminution occurred in response of the reticular formation or dorsal hippocampus to the avoidance CS, in contrast to the diminution observed in animals trained to the avoidance CR alone.

In studying the characteristics of electrographic data obtained during appropriate and erroneous behavioral responses to the two stimuli, it was observed that during appropriate response, potentials recorded from the specific sensory system and from nonmodality specific structures agreed with the frequency of the conditioned stimulus. When inappropriate response occurred, the frequency of potentials in the specific sensory structures still agreed with the conditioned stimulus. However, the potentials observed in nonspecific structures deviated from the presented CS frequency and tended to correspond with that of the stimulus appropriate to the behavioral response which was performed.

On the basis of these and other observations, these workers (57, 58) suggested that repeated experience with intermittent stimuli might result in the establishment of a neural system in nonspecific structures, with the capacity of discharging in a temporal pattern reflecting the temporal pattern of the conditioned stimulus responsible for establishment of the system. Discharge of this system with that temporal pattern might serve as a representation of a past event. The interaction of such representational systems with specific sensory systems in an inhibitory fashion might account for the suppression of repeatedly experienced input during habituation, and interaction in a facilitatory fashion might account for various phenomena of assimilation of rhythms, as observed during intertrial intervals or during generalization. Finally, congruence between the pattern of activity in this system and in the

specific system might constitute the basis of signal identification which would seem to be a logical requirement for appropriate performance of differential responses or discrimination.

In an elegantly designed experiment, Chow (21) analyzed the changes in visual and temporal cortex EEG during learning of a visual conditional reaction problem, in which the positive or negative significance of two patterns reversed depending on the frequency of the light flashes illuminating the patterns. Under these circumstances, certainly ideally calculated to maximize the frequency attribute of the stimulus as a characteristic of salient importance, Chow failed to detect any consistent marked changes in cortical EEG. The data suggest an increase in photic driving at the beginning of each frequency reversal and a rapid subsequent decrease. Further detailed study using this technique supplemented by quantitative data analysis methods and extensive recordings from subcortical as well as cortical areas should be of crucial importance.

In an interesting experiment described by Asratian (5), Sakhiulina elaborated a conditioned defensive reflex under so-called "switching" conditions. A left hindleg CR was evoked by the CS in the morning, and a right hindleg CR was elicited by the same CS in the afternoon. Recordings revealed a tonic focus of heightened activity maintained on the anterior parietal cortex contralateral to the operative paw.

Killam, Barlow, and Brazier (60) examined the effects of differential conditioning on the electrical response of the lateral geniculate to a flicker conditioned stimulus, using an average response computer. Establishment of a conditioned response to flicker altered the secondary response complex, and subsequent establishment of differential response to flicker of a different frequency was accompanied by a marked increase in complexity and duration of this secondary response. Brazier suggests this may be related to the need to match the stimulus with an internalized representation of the previous experience. Research of this sort, investigating the effects of experience on the waveform of evoked response in a structure, gives some indication both of the tremendous potentialities and the increasing indispensability of data processing computers in research on electrographic changes during conditioning.

If differentiation is considered as a process in which the generalization tendency to stimuli other than the conditioned stimulus is selectively inhibited by nonreinforcement of the differential stimulus (internal inhibition), an electrographic manifestation of such inhibition might be expected in the form of slow waves. Many studies of EEG activity during establishment of a differential response report slow-wave activity evoked by the differential stimulus (38, 40, 41, 89, 105, 113), although there is neither unanimity on this point (5, 21, 68, 112, 118), nor agreement as to persistence of this slow activity after differentiation is well established (40, 41). A number of these researchers presume the slow-wave activity to be the electrical manifestation of internal inhibition responsible for differentiation.

Basing their conclusions on various aspects of the data previously sum-

marized, but emphasizing the inhibitory nature of surface negativity arising from excitation of apical dendrites, Beritoff (9), Kogan (62), and Roytbak (94) propose that the essential mechanism of internal inhibition involves axodendritic processes which cause negativity of the apical dendrites of cortical pyramidal neurons. These influences are presumed to originate in the thalamic reticular formation; Beritoff suggests that a decrease in tonic excitatory influences exerted by the nonspecific system on associative or internuncial neurons might similarly interfere with the integration of stellate cell responses to stimuli.

#### EXTINCTION

Extinction of a conditioned response can be considered as a process essentially similar to that assumed to occur during differentiation—inhibition of a tendency to perform an act in the presence of a stimulus. This process is manifestly not the complete uncoupling of the temporary connection established by conditioning, since it is well known that an extinguished CR can be readily reactivated.

Lissák *et al.* (68) investigated differential effects of electrical stimulation of diverse structures on performance of previously elaborated approach and avoidance conditioned responses. On the basis of their results, they postulated two classes of mechanisms subserving internal inhibition: (a) differential inhibition, in which reciprocal inhibitory mechanisms of the brainstem and diencephalon predominate; and (b) extinction, in which the inhibitory mechanisms are primarily of hippocampal origin. Stimulation of the brainstem reticular formation or the hypothalamus revealed that a point facilitating one of the CR's always inhibited the other. Regardless of the direction of the effect, stimulation was invariably accompanied by diffuse cortical desynchronization. Slow waves were not observed during the development of behavioral differential inhibition. In contrast, hippocampal stimulation inhibited both types of CR's equally and independently of the stimulated point, and slow potentials appeared in the cortex like those seen during behavioral extinction. The hippocampus is proposed to have an aspecific inhibitory effect on the hypothalamus and the diffuse activating systems.

Killam *et al.* (60) report that the hippocampal response to the CS, progressively diminished during elaboration of an avoidance CR, returns while the CR is being extinguished, and attribute this to the process of attaching new inhibitory significance to the conditioned stimulus. The configuration of frequency-specific responses to the flicker CS, established as an avoidance CR was elaborated, diminished and disappeared parallel to the conditioned CR (56). Grastyán (41) also noted the reappearance of slow waves in the hippocampus, while Beck *et al.* (8), Roytbak (94), and Yuyama (121) all note the appearance of cortical slow waves during extinction. However, Shiliagina (106) and Adey (2) report the disappearance of slow waves from the cortex and hippocampus.

Jasper *et al.* (90) observed that grouped unit firing at the frequency of a flicker CS was seen in the motor cortex only during response failure or dur-

ing extinction [see also (122)]. Worden (113) similarly describes an increase in evoked response amplitudes during extinction of differentiated CR's, with response somewhat larger during the positive than during the negative conditioned stimulus.

Thus, although evidence seems to support the expectation of slow waves during extinction, the data are by no means in complete agreement on this point.

A summary of salient findings on which the previous discussion of process has been based, and additional data from the recent literature, organized according to functional systems, are presented in Table II.

### SUMMARY TABLE II

#### FINDINGS RELATIVE TO CONDITIONING PROCESSES, ORGANIZED ACCORDING TO FUNCTIONAL SYSTEMS

##### I. CORTICAL REGIONS

###### EEG STUDIES

*Early phase of conditioning.*—Generalized cortical desynchronization and widespread evoked potentials elicited by CS (5, 8, 27, 38, 56 to 59, 67, 71, 72, 90 to 92, 101, 103, 105 to 107, 110, 112, 117 to 121). Amplitude increase in all cortical layers (88). Unit activity in visual CS in visual cortex (76). During conditioned discrimination, transient increase in number of trials with reduced amplitude (21).

*Late phase of conditioning.*—Appearance of cortical slow waves (38, 59, 106, 120), or maintained desynchronization to CS (8, 91, 120, 121). Overtrained animals show localized arousal (8). Occurrence of driven rhythm decreases (67, 110, 122) but in other studies becomes more prominent (21, 56 to 58) or localized (72, 73). Changes marked in layer 4 of CS region and layer 5 of motor cortex (88, 94).

*Differentiation.*—Negative stimulus elicits generalized cortical depression (59) or desynchronization (112). Cortical slow waves observed (8, 38, 41, 94, 105, 112, 113, 120). Parietal cortex units firing at S+\* frequency inhibited during S-† (53, 90). When performance error in response to S-, same units fire as with S+ (90). With stable differentiation, S- most pronounced at layer 4 of CS area and in layer 5 of motor cortex (89). S- evokes S+ frequency pattern in sensory motor cortex (73).

###### STIMULATION STUDIES

*Elicitation and maintenance of conditioned responses, and cortical stimulation as a CS or US.*—Inhibition of CR as consequence of localized convulsive discharge of CS cortical region but not of US region (122). Central stimulation in rat effective as CS with CER‡ but not in CAR§ (74). Effective as CS for CAR in cat (80). External inhibition (to loud sound) demonstrated when CS used is central stimulation (99). CR elaborated with CS and US central stimulation (27). When central stimulation is CS, transfer occurs between cortical CS and peripheral stimuli of same modality and between homotopic cortical points (25). Undercutting cortical stimulation area eliminates CR; circumsection does not (24). When motor cortex stimulation is US, peripheral CS causes decrease in threshold at reinforcement point and increase elsewhere late in conditioning (62). DC polarization or tetanizing stimulation alters response (65, 78, 89, 93, 96 to 98, 109, 117). No disruption of acquisition or retention of discrimination reversal (84). Spreading cortical depression inhibits CR's (14 to 16).

\* S+ = positive stimulus.

† S- = negative stimulus.

‡ CER = conditioned emotional response.

§ CAR = conditioned avoidance response.

(Summary Table II continued on pages 478-479)

## SUMMARY TABLE II

FINDINGS RELATIVE TO CONDITIONING PROCESSES, ORGANIZED  
ACCORDING TO FUNCTIONAL SYSTEMS

## II. HYPOTHALAMUS

## EEG STUDIES

*Early phase of conditioning.*—Alimentary CS elicits (a) increase in frequency of medial and lateral hypothalamic activity (71); (b) evoked responses (57).

*Late phase of conditioning.*—Increased frequency shifts toward CS onset with no difference between medial and lateral structures (71). Frequency-specific CS represented in region where self-stimulation rate covaries with hunger (83).

## LESION STUDIES

*Retention.*—Section of the mammillothalamic tract results in loss of CAR and difficulty in reattaining criterion (111). Lesions involving mammillary bodies, field H<sub>1</sub> of Forel, centromedian nucleus and habenulopeduncular tract impair CAR retention (26). Destruction of posterior hypothalamus does not affect retention of CAR (26).

## STIMULATION STUDIES

*Elicitation and maintenance of conditioned responses.*—Stimulation of various hypothalamic nuclei elicits CR's (3, 39, 68, 114). Stimulation cessation can act as partial reinforcement (116). Stimulation at same point can motivate either approach or avoidance CR's (13). DC polarization alters response (97). CR established if conditioned movement occurs during stimulation but not if before (115).

## III. THALAMIC STRUCTURES

## EEG STUDIES

EEG arousal is present in animals with extensive lesion of rostral thalamus (20).

*Early phase of conditioning.*—CS elicits generalized desynchronized fast activity (103, 120) and regularized rhythm in medial thalamus (107), and slow activity in CL|| (105).

*Late phase of conditioning.*—VA# response to the CS appears (56). Initial desynchronization is replaced by 4 per sec. slow waves (118, 120). Increased response in CL (118). Increase in evoked potentials during approach conditioning (57). VA unit discharge increase during final stage of cortical conditioning (76).

## LESION STUDIES

*Acquisition of CR.*—Visual pattern discrimination learned following lesions of rostral pole (20). CM\*\* lesion blocks development of cortical conditioning (119).

*Retention of CR.*—CM may be involved in the retention of CAR (26). CM lesion following conditioning of the effects of CM stimulation impairs retention of this CR (103).

## STIMULATION STUDIES

*Elicitation and maintenance of conditioned responses.*—Lateral and medial thalamic stimulation inhibits CR (30). Stimulation of CM in conditioning environment evokes elaborated CR (30). CR established to tone using CM stimulation as US (103). CM stimulation causes a cortical DC shift which can be conditioned (76). CM stimulation can be used as US in cortical conditioning (118). Midline thalamic stimulation prevents learning but not retention (83).

## IV. MESENCEPHALIC RETICULAR FORMATION (MRF)

## EEG STUDIES

Stimulation of points facilitating CR's elicits diffuse desynchronization (39).

*Early phase of conditioning.*—During initial stages CS elicits (a) small-amplitude slow potentials (41, 120); (b) enhanced evoked potentials (56, 117), or regularized rhythm (107). MRF units show increased firing to both CS and US (76).

|| CL = nucleus centralis lateralis.

# VA = nucleus ventralis anterior.

\*\* CM = centromedian nucleus.

## SUMMARY TABLE II

FINDINGS RELATIVE TO CONDITIONING PROCESSES, ORGANIZED  
ACCORDING TO FUNCTIONAL SYSTEMS

*Late phase of conditioning.*—During stage of well-established CR the CS elicits (a) slow waves (120); (b) diminution or disappearance of evoked responses (56, 76, 103) more marked in CAR (56) than approach training (57,58). Terminal phase of cortical conditioning manifests a decrease in MRF unit firing (76).

*Differentiation.*—Negative stimulus elicits irregular slow waves (105).

## LESION STUDIES

*Acquisition.*—Animals with extensive bilateral MRF lesions can learn pattern discrimination, defensive reflex, and CAR (23, 26); also manifest EEG arousal (36, 70).

*Retention.*—CAR or conditioned defensive reflexes retained after MRF lesions (23, 26, 63). MRF lesions do not impair retention of a CR involving MRF stimulation as the US (103).

*Differentiation.*—No disturbance of a differentiation with MRF lesion (26, 63), but chlorpromazine effects potentiated (63).

*Additional.*—Extensive two-stage bilateral MRF lesions do not abolish alertness (1).

## STIMULATION STUDIES

*Elicitation and maintenance of conditioned responses.*—MRF stimulation applied alone in the CR environment elicits the complete CR (30, 39). MRF stimulation ameliorates the inhibition of CR's produced by conditioned "neurotic" behavior (30, 122). Facilitates differential learning (122). CR to tone can be established using RF stimulation as the US (103). No disruption of acquisition of discrimination reversal (84).

## V. HIPPOCAMPAL SYSTEM

## EEG STUDIES

*Early phase of conditioning.*—Response to CS consists of rhythmic slow waves (2, 40, 41, 120). More prominent in dorsal hippocampus while ventral resembles neocortex (2, 41). CS elicits evoked potentials (33, 43, 56, 67, 117). Units respond to both CS and US in first stage of cortical conditioning (76).

*Late phase of conditioning.*—CS evokes desynchronized activity (40, 41). Previously observed evoked potentials are diminished or eliminated (43, 67, 76, 119). This decrease in evoked potentials is differential, appearing in avoidance (56) but not in approach CR's (57). Dorsal hippocampal units no longer respond to either CS or US (76), during cortical conditioning. Different parts of hippocampus show changes in phase angles from early to late training (2).

*Differentiation.*—At early stages of differentiation the S- elicits rhythmic slow potentials. After elaboration of the differentiation, the S- elicits desynchronization (40) and finally exerts no influence (41). At the beginning of differentiation, S+ elicits hippocampal slow waves. This changes to hippocampal desynchronization and short-latency CR's (40). Differential conditioning stabilizes frequency-specific response patterns (56).

## LESION STUDIES

*Acquisition.*—Bilateral hippocampal lesion blocks CAR acquisition in rat (61) but not cat (40, 51). This effect appears to be restricted to posterior portions in the rat (61).

*Retention.*—CAR retention is impaired to an auditory CS but not to a visual CS unless the auditory is overtrained (51).

## STIMULATION STUDIES

*Elicitation and maintenance of conditioned responses.*—Previously elaborated approach and avoidance CR's are absent during hippocampal seizure (32, 40, 68). Cortically evoked motor responses are also inhibited during the after-discharge (32). Cats trained during hippocampal after-discharge retain CR's when tested after cessation of after-discharge despite failure to evoke response during this interval (32). Stimulation of the hippocampus at a frequency equal to a peripheral CS evokes CR after a period of pairing both stimuli (67). Hippocampal stimulation interferes with acquisition of CR (84) in rat.



## SUMMARY

Having surveyed a substantial portion of the recent data made available by the application of chronic stimulation and recording techniques to the problem of conditioning processes, we may now ask what formulation can be proposed to reconcile these data with each other and with information from ablation studies? On initial consideration, the descriptions of processes which emerge from electrographic studies seem incompatible with the relative lack of effects on conditioned response acquisition and retention reported in studies involving lesions. Regions which seem intimately involved in CR mediation can be damaged severely without preventing CR acquisition or retention.

For example, electrographic evidence has led numerous workers to attribute a central role in CR elaboration to the brainstem reticular formation. Yoshii (118), Gastaut (34), Majkowski (72), and Roytbak (94) have suggested that the RF is involved in initial stages of connection formation. Yoshii and Gastaut propose that general desynchronization produced by the CS early in conditioning indicates participation of the mesencephalic RF, while the subsequent local desynchronization in later stages of conditioning shows participation of the thalamic reticular formation. Maeno (105) proposed that internal inhibition results from suppression of the reticular activating system. Grastyán (41) suggests that such RF inhibition arises via hippocampal activation. Yoshii (118) concurs and proposes that these hippocampal influences, mediated by the thalamic RF, may exert their final inhibitory effects via a corticoreticular projection.

In view of this general consensus, recent findings of Chow (23) seem at first to be startlingly incongruous. This inheritor of the tradition of Lashley has reported that cats with massive bilateral lesions of the reticular formation not only show EEG and behavioral arousal, confirming other findings (1, 70), but can acquire visual pattern discrimination and avoidance conditioned responses or retain these if established before surgery. Need we be disconcerted by such findings? The fact that an animal can learn or retain a response after a lesion does not of itself warrant the conclusion that this structure normally plays no role in the response. The engram, we suspect, is wily enough to elude the subcortical shot as easily as the cortical knife. Memory seems more likely to be a set of processes, which define a state, than a "bit" in a place. That places participate in process is apparent, but we may more legitimately expect the lesion of a region to alter the process than to abolish the state. That lesions can alter process we know, witness the effect of septal lesions on slow hippocampal waves. The problem that confronts us is to unravel how the conditioned response is produced by the process.

To this reviewer, electrophysiological observation seems to provide the most intimate insight into process. Intensive effort to analyze how the process produces the conditioned response can proceed by use of tools already on hand. The brain with a lesion must perform differently from the intact brain. Studies of the effects of lesions, of central stimulation, of drugs, of polariza-

tion, are currently being performed in animals which are learning and performing while implanted gross and microelectrodes provide information about changes in process. The results of studies such as these promise new insight into the old paradox.

The data already on hand support portions of earlier conceptions but suggest the need for some modifications and extensions. During initial presentation of a novel stimulus, both excitatory and inhibitory effects are widespread throughout the brain. These effects diminish and disappear with repeated inconsequential experience, in a fashion which suggests a selective inhibition. With reinforcement, response reappears. Irradiation does occur, and signs of hippocampal and mesencephalic reticular formation involvement appear.

Evidence exists both for and against consolidation. Evoked potential data generally indicate that consolidation occurs, while desynchronization criteria apparently oppose this conclusion. Further research is needed to establish whether this apparent contradiction arises from the interpretation of desynchronization as activation when it may, in places, be inhibitory. Evidence exists further for two reciprocally inhibitory arousal systems, one related to food-getting and the other to defense. The rate of consolidation may depend appreciably on the relevant drive.

Three factors may account for the apparent reversal of consolidation with differentiation. (a) Rebound effects may arise from interactions between these reciprocally inhibitory systems, as positive and negative stimuli are presented. (b) A differential discrimination may require the comparison of a stimulus with a representation of past experience, in contrast to an existential discrimination, probably based on the presence or absence of the stimulus. Evidence exists for such representational systems. (c) During differentiation and extinction, performance of the conditioned response elicits a novel and unpredictable consequence—nonreinforcement. Excitatory sequelae to this new event, as well as inhibitory influences on the unreinforced process, might be the source of the superficially paradoxical reports of both hypersynchronous slow waves and desynchronization or increased evoked potentials during these procedures.

These generalizations would be more numerous and more positively asserted if electronic data analysis equipment were more generally available, and if research reports provided more detailed information about the events which ensue during the various stages of learning.

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## HEARING<sup>1,2</sup>

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The search for published literature related to the physiology of the ear is like beating the dust out of a rug; one never seems to find an end-point. As a matter of fact, it is a rather unsatisfactory feeling to stop at last and write a review claiming to have covered four years of the publications on hearing, just as unsatisfactory as putting that still-dusty rug back in the house. At the very outset it must be stated, and with certainty, that no topic is completely covered nor, for that matter, are all the topics related to the subject touched upon. Consolation lies in the thought that this review is to furnish leads to the publications in some areas and that the interjected ideas are there to stimulate other ideas that may not, as yet, have been expressed.

There have, of course, been many related reviews in the last four years and this makes the selection of material for this review somewhat easier. As the topics are covered, any general review that has not been missed by the reviewer will be mentioned and only pertinent articles discussed.

Two general reviews have appeared in the *Annual Review of Psychology*, one in 1958 by Harris (47) and one in 1959 by Jeffress & Moushegian (50), that contain discussions of the anatomy and physiology of the ear. In a comprehensive chapter on "Excitation of Auditory Receptors," Davis (17) has brought together much that relates what is known about the mechanical action of the inner ear, in response to sound, to the electrical phenomena occurring both in the sensory area of the ear and the nerve pathways; he has also covered this in a much more detailed manner in another review (15).

No doubt the biggest event since Helmholtz published his *Sensations of Tone* has been the appearance of the book by Békésy (8) which contains translated and edited (by E. G. Wever) reprints of all the publications related to hearing that Békésy had ever published up to the time of issue of the book. He has organized this tremendous and invaluable material under certain subject headings, a procedure which has broken up the chronology but sorted the material for the reader. There are four major parts: the Introduction covers problems, anatomy, and experimental apparatus (and here Békésy is a genius); Conductive Processes covers the middle ear and bone conduction; The Psychology of Hearing covers auditory thresholds, spatial attributes of sound, and problems of distortion; and Cochlear Mechanics covers the areas for which Békésy is best known: patterns of vibration in the

<sup>1</sup> The survey of the literature to which this review pertains covers the period July 1, 1956 to July 1, 1960.

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cochlea, wave motion in the cochlea, frequency analysis, and the electrophysiology of the cochlea. This is a book that no serious student of hearing should be without.

One more book worthy of mention concerns the fundamentals, use, and design of electronic apparatus for biological research, by Donaldson (26). This is well organized and well written and contains much that the researcher would like to know. Its four parts cover theory, practice, transducers and electrodes, and complete apparatus. Electronic techniques are changing so rapidly that it is difficult for one to keep up with them; however, with a book like this at hand, the distance one falls behind can be minimized.

#### THE MIDDLE EAR

*Surgical alteration.*—The big news in middle ear physiology lies in the tremendous advances made in middle ear surgery for the relief of deafness associated with diseases of the tympanic cavity. The big strides in medicine are always those that produce a complete understanding of the etiology of a pathological condition and lead to its alleviation through surgery or medicine based on equally well understood physiological principles. After about three-quarters of a century of experimentation designed to determine middle ear function, the principles were established and described and the otologic surgeon was able to reconstruct successfully with a definite plan the diseased middle ear structures and so relieve this type of deafness. (The use of antibiotics for the control of infection has made much of this possible.)

Although operations on the inner ear were attempted in the 1890's with few ill effects, Shea (91) has successfully restored hearing in many cases to normal by an amazing operation. He opened up the vestibule (with loss of perilymph) by removing the footplate of the stapes, covering the opening with a bit of vein and replacing the stapes with a plastic prosthesis. Most investigators have felt that opening the inner ear would eventually cause its deterioration through the loss of fluids, but apparently this is not always so and such maneuvers stir one's imagination concerning the future treatment of other forms of deafness. For a review of present-day procedures, the reader is referred to the beautifully written book by Shambaugh (90). Other discussions appear in the posthumously published book by Kobrak (55).

It appears that the endolymphatic system will function very well even with much of the perilymph of the vestibule drained out [Lawrence, Burton & Wolsk (62)]. All that is necessary is to have two open windows on opposite sides of the scala media so that mobilization of the cochlear partition can occur.

A point of interest that has arisen through these surgical manipulations of the middle ear is that raised by Rosen (87) who claims to have restored normal hearing in an otosclerotic ear merely by the process of puncturing the footplate of the rigidly fixed stapes. He feels this indicates that the transformer action of the ossicular chain is not necessary to produce normal hearing. His observations do raise a question, but careful experimentation in animals by Fernandez *et al.* (35) has failed to confirm his hypothesis. Probably



the attempt to perforate the footplate has merely broken it free, thus remobilizing it to produce a normal situation.

*Impedance measures.*—Much of the success of middle ear surgery depends upon the proper diagnosis of the type of hearing impairment. Comparison of bone-conduction and air-conduction thresholds has been the traditional measure, but bone conduction occasionally is unreliable, and an objective method for determining the exact extent of ossicular immobility that is causing the conductive deafness would have great value. [A good review of bone conduction is given by Kirikae (54).] A procedure that is being investigated very sparsely but which is gradually making progress is that of measuring the impedance of the ear. Older experimental methods employed an acoustic bridge that was rather cumbersome and difficult to fit securely into the external meatus. Recently Zwislocki (116, 117) has described a smaller model and used it for some preliminary measures in normal and pathological ears. The method still gives highly variable results, but there is no doubt that pathology of the middle ear influences the impedance.

Another method, perhaps even more sensitive, was described by Møller (77). This is a device that is compact enough to be inserted into the external meatus and supported by a band on the head. His measurements were made on normal hearing subjects only, and again the spread of data proved to be extensive. Perhaps this spread is real, but at any rate the miniaturization of the device is progress and may some day be of great aid to the clinician.

*Middle ear muscles.*—The stapedius muscle is generally cut during the course of surgery upon the middle ear structures, and questions are often raised concerning the effect this might have upon hearing. In the past it has often been reported that the majority of patients suffering from facial palsy and a consequent paralysis of the stapedius muscle experience what is commonly called "hyperacusis". There has been some debate over this term because literally it means an abnormally acute sense of hearing, but, by general use, it has come to mean an abnormal feeling of discomfort produced by sounds above threshold intensity.

Two explanations have been given for this phenomenon: one attributes it to a mechanical effect of muscle paralysis, the other to the cochlea, giving rise to the auditory experience called recruitment. Recently some experiments have been performed that seem to answer the question.

Ruben *et al.* (88) cut the stapedius muscle while recording the cochlear potentials in man and found a change of from 36 microvolts to 42 microvolts. Although there was no standardization of the sound stimulus and the increase amounts only to 1.5 db, there is the indication that for a given sound input to the ear the sound is louder when the stapedius is cut.

Galambos & Rupert (42), on the other hand, presented results that seem to indicate the opposite effect. These investigators prepared a number of cats with implanted electrodes on the round window of each ear. On one side the two intratympanic muscles were cut and on the other the middle ear was left intact. These ears were stimulated by sound and the electrical response of the ear recorded by photographing the tracings of a cathode-ray

oscilloscope. From their experiments, Galambos & Rupert concluded that in the unanesthetized animal the muscles provided no mechanical support to the ossicular chain and exerted a negligible influence upon sensitivity. As the sound intensity is raised, the round-window responses were reported to differ markedly in the ears with intact muscles from the responses in the ears with muscles cut. These workers were unable to separate the factors responsible for the differences, but the published cathode-ray oscilloscope pictures show the initial responses of the ear with muscles to be greater than the response from the ear with cut muscles (assuming the amplifier gains to be the same).

Reporting from the same laboratory but much more extensively, Simmons (92) presents results on cats with implanted electrodes that definitely showed a response to a given sound pressure to be greater when the stapedius muscle is cut than when it is present. Similar results are reported by Lawrence (61). Apparently the middle ear muscles provide a damping influence upon the vibrations of the ossicular chain and protect the inner ear from excessive amplitudes of vibration associated with the higher sound intensities. Loss of these muscles may bring about the abnormal discomfort to sounds at moderate levels (hyperacusis).

Other studies of the muscle reflex have revealed that hearing loss from impact noise (machine-gun fire) can be lessened by initiating the muscle reflex by a brief burst of tone before the impact noise: in man by Fletcher & Riopelle (39), and in cats by Hilding (49). A comprehensive review of past studies on the middle ear muscles was presented by R. Wersäll (107).

#### ANATOMY

*Cochlear duct.*—Despite the recent spread of interest in the research potential of the electron microscope, the light microscope has its place in teaching anatomy, pathology, and research on "gross" microscopic changes under certain experimental conditions. A real aid to the teaching of temporal-bone anatomy has appeared in the book on microscopic anatomy of the temporal bone by Wolff, Bellucci & Eggston (113). Progressive sections are pictured with labels for the three different planes of section commonly used. Attempting to put some bit of predictability in the sequence of autolysis seen in temporal-bone sections, Fernandez (33, 34) compared in one study the appearance of the tissues after three different fixation techniques: removal of the stapes before immersion in fixative, routine immersion, and injection of fixative into the middle ear cavity soon after death. Removal of the stapes proved to be no more advantageous than the other techniques. Injection of formalin into the middle ear soon after death gave a higher proportion of good results than immersion procedures. In the other study, intravital perfusion, post-mortem perfusion, and post-mortem immersion were compared. No procedures preserve the tissues quite as well as intravital perfusion.

One of the reasons we still do not know completely how the ear works is because we do not know the details of inner ear structure, and even if we did it would still be necessary to determine the functional significance of the various parts. Many theories of hearing have been based upon the existing

information; others have postulated the existence of certain structures. Thus Helmholtz (48) suggested a series of tuned resonators along the cochlear duct, but subsequent careful study of the organ of Corti has failed to reveal such structures. The theory most widely accepted today (see discussion under cochlear mechanics) calls for a mysterious interplay of events between hair cells and terminal nerve fibers at the initiation point of the nerve impulses. Many of these fibers have been described, but some may be beyond the reach of the light microscope. Also, the fine structural details of the sensory and supporting cells, as well as the details of the nerve to sensory cell connection, are at the limits of resolution for the light microscope. A new world has opened up with the use of the electron microscope, and we find the small structures of the ear being re-examined in considerable detail by this relatively new technique.

Jan Wersäll (106) presented a review that gives leads to the previous literature as well as describing some of the techniques. This appeared in 1956, and much has been done since. A later (1958) review by Engström & Wersäll (31) is quite thorough. They present details of cell structure and nerve endings and give many references.

It is a most rewarding area of research; one only needs access to an electron microscope, a little skill and patience, and some hitherto unexamined tissue. The ear, with six different sensory areas, is a gold mine. The big drawback is the inaccessibility of the material, especially human, which so far has not been touched.

Smith (94) has subjected the structures of the stria vascularis and spiral prominence to this highly magnified scrutiny. The major cells of the stria are those facing the endolymph, and the basal plasma membrane has many folds and projections especially around the capillaries. She points out the similarity of these structures to those found in kidney tubule, serous alveoli, and secretory duct of the submaxillary gland, all of which are noted for their fluid transport, suggesting that the same function may be served in the membranous labyrinth.

One difficulty with electronmicroscopy is that such very thin sections have to be cut that it is difficult to cover much of a region through a block of tissue. It seems to be well established [Lawrence (57)] that the area of the spiral prominence has structures interspersed among other structures and that one would get a different kind of cell, depending upon which area happened to fall under the microtome knife. Further studies of the spiral prominence with the electron microscope should reveal these. The source and resorption of inner ear fluids are still a real mystery. Every bit of evidence seems to make the problem more confused, but correlation of studies on structure and function may eventually turn up some answers.

*Inner ear fluids.*—Attempts to determine the chemical constituents of the inner ear fluids is complicated because there is never very much available and it is not easy to keep one fluid from contaminating the other. Antonini, Casorati & Crifo (1) solved the problem of quantity by combining samples of perilymph obtained from a number of horses. From the round window of

each animal they could obtain from 0.015 to 0.025 ml. of perilymph. They determined the protein content of this fluid by paper electrophoresis and compared it with cerebrospinal fluid and blood serum. The protein content of the perilymph appeared to be greater than that of cerebrospinal fluid but less than blood serum.

The vitamin content of horse perilymph was determined by Ferrari & Casorati (38). There have been speculations in the past on the role of vitamins in the ear which makes their actual presence important. No vitamin A or B<sub>6</sub> was found in the perilymph, but B<sub>2</sub> and a coenzyme derived from it, together with a reduction substance most probably connected with vitamin C, were found.

The problems involve not only the contents of these inner ear fluids but also their location. Rauch & Köstlin (86) have reviewed in table form, called ionograms, the contents of endolymph and perilymph. In a later paper, Rauch (85) concludes that the hair cells are not surrounded or nourished by endolymph, which is not a new notion, having been brought up before because of the high potassium content of the endolymph.

Tasaki, Davis & Eldredge (101) decided that the high potassium content, would, if present around the hair cells and their associated nerve fibers, surround the sensory endings with more than enough potassium ions to abolish the electrical responses completely. They maintain that the organ of Corti is actually a part of the scala tympani and that the membrane separating the perilymph of the scala tympani from the endolymph of the scala media is not the basilar membrane but is the reticular membrane, the supporting structure for the hair-bearing ends of the hair cells. This is more or less what Rauch also says.

These speculations raise a number of questions such as: why is there a decrease in local electrical activity of the organ of Corti when perilymph or a synthetic substitute is allowed to mingle with endolymph in the scala media?

One way to answer the question of location of inner ear fluids is to stain the tissues with a dye that would react differently to the contents of the fluids that remain in the ducts after the histologic processing. Lawrence (60) has presented some results, using such a stain on guinea pig ears, some of which were normal and others that had been overstimulated by noise of sufficient intensity to rupture Reissner's membrane. The endolymph in the normal ears and in those portions of the scala media where Reissner's membrane was not broken stained darker than did the perilymphatic spaces. The dark stain included the organ of Corti down to the basilar membrane, and it was concluded that the boundaries of the scala media and its contained endolymph are the limbus, Reissner's membrane, stria vascularis, spiral prominence, external sulcus, basilar membrane, and internal sulcus.

An important observation was that only in those regions where Reissner's membrane was broken did the two fluids seem to mingle, even though these animals were sacrificed two months after the overstimulation that had ruptured the membrane. That one part of the membranous labyrinthine sys-

tem can be injured without necessarily causing injury in other places has been reported by Kristensen (56) and Borghesan (12) and by others in earlier years. The number of observations that do not fit together makes a theory of cochlear and vestibular fluid flow difficult to piece together. The many variations fall into two general classes of theory: one supposes that the endolymph flows longitudinally down the cochlear duct to the endolymphatic duct and sac; the other expresses the notion that fluid flow is radial, being secreted and absorbed in more or less the same area along the duct. Experiments on restricted local damage seem to support the latter, but dye injection experiments point to a dynamic longitudinal fluid flow. Other recent experiments of this sort have been carried out by Van Egmond & Brinkman (104) and Svane-Knudsen (100); neither obtained very conclusive results.

These dyes, carbon particles, etc., act as foreign substances within the cochlea and give information only about what the labyrinthine system does with such material. Now that isotopic tracer elements are available, it is possible to inject tagged substances that are normal bodily constituents. These can be followed throughout the tissues, and if they are taking part in the metabolic processes of certain cells or tissues their eventual fate can be determined.

Dohlman, Ormerod & McLay (25), Dohlman & Ormerod (24), and Dohlman (23) have described experiments using this technique. Injections of radioactive sulfur ( $S^{35}$ ) were made intramuscularly in pigeons. The birds were sacrificed at different intervals of time after the injections and the ears processed as usual for histological study. Appropriate sections were covered by a film in a dark room, and after a suitable length of time exposed spots appeared on the film wherever the radioactive element was located in the tissue. When the photographic film with the thin section attached was studied, a good picture was obtained of the location of the tracer element and if this element took part in a metabolic process some idea could be obtained about the metabolism. Dohlman finds that the important constituents of vestibular and cochlear metabolism—sulphomucopolysaccharides—are secreted in the "appropriate epithelium". In the cochlea he indicates that this epithelium is in the spiral ligament extending beyond the stria vascularis. From studies of birds killed when more time had elapsed since the injection, Dohlman concludes that the radioactive sulfur passes from the spiral ligament directly to the tectorial membrane where the substance is taken up. He found no evidence that the tracer elements entered the sensory elements; so he concludes that the tectorial membrane is the seat of metabolic activity. The hairs of the sensory cells are described as lying in tubular canals within the membrane where the metabolic exchanges take place. The electrical potentials of the cochlea, Dohlman believes, arise from the condenser action of the mucopolysaccharides and potassium on the membranes of the hairs and cells.

Portmann *et al.* (83) used radioactive sodium ( $Na^{24}$ ) injected into the blood and traced the relation between this element in samples of perilymph as compared to blood. In perilymph the sodium level rises rapidly during the

first five hours and then levels off. This experiment would have great possibilities if carried further.

Interesting theories of endolymph circulation, too lengthy to review here, have appeared. Evidence is beginning to favor the radial flow class of theories and some seem fairly plausible. Borghesan (10, 11) proposed a radial theory starting from the premise that endolymph cannot be secreted by the stria because its histological structure is "inadequate". He believes the spiral prominence to be the source.

A fascinating review of the literature and an interesting theory which could serve as the basis for experimentation are presented by Naftalin & Harrison (78). The problem of endolymphatic and perilymphatic circulation seems to be attracting more attention, probably because of the new techniques now available, and there is still much to be learned.

*Terminal nerve fibers.*—There has been considerable interest in the last few years in the nature of the terminal nerve fibers within the organ of Corti. This has been true partly because of the relation these structures might have to auditory theory and also because of the intriguing challenge to bring about a more thorough understanding of the efferent system to the organ of Corti. Portmann (82) and Contu (14), using silver stains, have both given excellent pictures confirming some of the older descriptions. Interesting statements were made by Engström in the discussion of Portmann's paper. Engström points out that the diameter of the ramifications of the cochlear nerve within the organ of Corti may have a diameter of about 0.2 micron or even less and that only with a heavy precipitation in and around such nerve fibers could they be seen with the light microscope. The electron microscope, of course, could give a better picture and recently Engström (30) has presented this material. There is good evidence for two distinct, different structural systems of nerve terminals within the cochlea as well as within the vestibular sensory areas. One of these systems of nerve terminals has characteristics of postsynaptic fibers and the other of presynaptic fibers. One of these is probably the efferent system and Engström discusses the evidence for and the consequences of such a system.

Other reports have appeared testing the functional significance of these fibers. Galambos (40) found that the auditory nerve discharge to a click stimulus of weak or moderate strength was reduced or abolished by electrical stimulation of the floor of the medulla at the site of the decussation of the olivocochlear pathway. These fibers originate in the superior olivary region and terminate on the hair cells (Engström's evidence) in the contralateral cochlea. In another report (41) Galambos ties this system in with responses from an "attentive" and a "nonattentive" animal. These are interesting leads but, of course, only the beginning, for this is an extremely complicated process tied in with brain function in general.

Schuknecht, Churchill & Doran (89) present another line of evidence. They perfused fresh cat cochleas with acetylcholine in the presence of cupric ions. In some cats the olivocochlear tract had been sectioned in the medulla and upon histological examination the cochleas showed heavy de-

posits of copper sulfide resulting from activity of acetylcholinesterase. Other cochleas from control cats did not show this. These workers conclude that the presence of acetylcholinesterase in the cochlea is dependent upon the integrity of the olivocochlear bundle, and they suggest that acetylcholine liberated at the endings of the olivocochlear tract fibers at the hair cells may alter their excitability or that of the acoustic nerve endings.

The neural supply to and from the cochlea is proving to be most complicated; and before a true explanation of end organ activity can be given, not only the anatomical details of the terminal fibers have to be worked out, but also their relation to the actual mechanical operation of the receptor structures within the cochlear duct. Engström has concluded from his electron-microscopic work described above that because one type of fiber with large richly granulated nerve endings terminates below the first row of outer hair cells, there must be a functional difference between the different rows of outer hair cells. This comes close to the suggestions of others who have attempted, by different experimental techniques, to understand cochlear mechanics.

#### COCHLEAR MECHANICS

*Frequency and intensity distribution.*—The chief investigator in the nature of mechanical action within the cochlear duct has ever been Békésy. In a series of papers (4 to 7) he has proposed two very sound ideas based upon experiments with what he has called a "model of the cochlea with nerve supply", referring to a special arrangement of stimulators and the skin, and supported by psychophysical experiments on hearing itself. One of these principles he has called "neural funneling" and the other, which really depends upon this funneling, is the co-ordinate system of analysis among hair cells: tones are separated according to their pitch along the length of cochlear partition and according to their loudness along the width, i.e., from outer to inner hair cells. That the inner and outer hair cells differ in sensitivity has been indicated for some time on the basis of stimulation deafness experiments and also falls in line with the suggestion made by Engström on the basis of terminal nerve supply.

The concept of funneling is proposed in addition to the accepted physiological events at a synapse of summation and inhibition. In funneling, summation and inhibition occur at the same time in the sense organ. This is best demonstrated by Békésy's experiment in which five vibrators were placed two centimeters apart along a line on the skin of the forearm. The amplitude of each vibrator was so adjusted that they felt equally intense when each was presented alone. The frequency of each was increased in one-octave steps from the upper arm to the wrist. When all five vibrators were vibrated simultaneously, only the one vibrator in the middle was felt with a subjective frequency corresponding to the physical frequency. The different frequencies of the other vibrators were lost, but their presence did contribute to the "loudness" of the middle vibrator.

Enlarging on this, and after considerable experimentation, Békésy points



out the two results of any local stimulation. There is (a) a local excitation and (b) a depression of the sensitivity and transmission ability all around the excited area. One can easily see the implications for auditory theory. The concept of funneling answers the problem of how the first steps of peripheral frequency analysis take place. The response of the basilar membrane to sound, although its region of activity moves from apex to base as frequency is raised, is made with a fairly broad region being activated. The funneling properties of the terminal nerve fibers narrow this action down to some center frequency. The long-sought-for sharpening mechanism is not strictly a property of the basilar membrane and organ of Corti but is a product of the interaction of a mechanical pattern of stimulation and a neural funneling action taking place among the terminal nerve fibers.

The other aspect of the co-ordinate system that refers loudness to a radial distribution is not so clearly defined, but the evidence is quite clear that there is a distribution of sensitivity as one proceeds from the outer hair cells to the inner ones. In the final analysis, the response system must be quite complicated, and Békésy has never attempted to translate the mechanical movements into all of the electrical events that it is possible to record by intracochlear electrodes. However, this has been done quite extensively by Davis and his co-workers (16, 18). The most thorough attempt to tie all known events together can be found in the classic review by Davis on the "Biophysics and Physiology of the Inner Ear" (15). Because of this review no attempt to describe the events will be made here, but the subsequent development of the funneling principle can now be added to the description.

When place theories without sharp resonance were designed, it was found that the pattern of vibratory action along the basilar membrane was too broad; so several mechanical sharpening mechanisms were proposed [see Wever & Lawrence (110)]. One of these was suggested some time ago by Békésy who noticed that the pattern of vibration caused eddies to occur in the fluid of the scala media. These eddies were supposed to reflect back upon the organ of Corti and do the stimulating of the hair cells. With the concept of neural funneling at hand, such retroactive events are not necessary to explain "sharpening" and are probably just a coincidental event, the very movement of the organ of Corti that has stirred them up having already stimulated the sensory cells. There is no question that eddies do occur, and Tonndorf [(102) and later articles] has made an extensive study of them in models of the cochlea, but as Davis & Eldredge have said, their generation will probably follow the vibratory action as a simple corollary. Other models have been built and studied to see if they behave according to the builder's concept of the ear, and they do. [See Meyer (71) and Bogert (9).]

*Aural harmonics.*—One of the amazing attributes of the ear is its extensive intensity range. Even the outer-inner hair cell sensitivity differential does not seem to account for this, and yet, as Stevens (99) demonstrates, it is probably a property of the end organ. When electric current is applied directly to the nerve endings such as in the skin, the range of tolerance for current increase is very small, yet when stimuli pass through an appropriate

end organ the range is great. When noise generated in one ear by an electric current is matched in loudness with an acoustically produced noise in the opposite ear, the growth of loudness is many times steeper in the electrically stimulated ear than in the one that is acoustically stimulated. Something must be happening in the organ of Corti that has not yet been accounted for.

When the electrical AC potentials of the cochlea are measured in response to increasing sounds, they are found to cease responding linearly with increasing stimulus intensities. As this occurs, harmonics are generated which can also be recorded if suitable filters in the pickup equipment are used. An experienced listener can hear these aural harmonics, and their audibility is enhanced by the use of an exploring tone which beats with the aural harmonic. Lawrence & Yantis (65, 67) have used this technique for an extensive study of the onset and growth of the aural harmonic. Békésy (3) and others have argued against the validity of using an exploring tone, but Lawrence (58) listed the reasons why its use can be considered as practical, and Yantis & Magielski (114) showed the clinical applicability of aural harmonic measurements.

The physiological question concerns the source of these aural harmonics. At the low levels at which the harmonics first appear, it has been shown that they do not arise in the middle ear. If these distortion products arise in the inner ear, is their generation caused by some mechanical nonlinearity of the sound conduction pathways or by some event within the organ of Corti? This problem is not resolved; there are experiments that seem to present evidence for both sources. Tonndorf (103), using electronic equipment capable of "delivering approximately 130 db undistorted output" (sound pressure level), concluded that harmonics are generated hydrodynamically and produce a traveling wave of their own so that they stimulate the same area of the basilar membrane that they would if they were a fundamental. On the other hand, Six (93) and deBoer & Six (22), using equipment for which no sound calibration was made, found the potentials of the combination tones to originate at the place in the cochlea where the primary tones give the maximum electrical effect. There have been other experiments related to this problem but none of them has yet solved it.

A phenomenon, called remote masking, whereby the threshold for low frequencies is raised when a high-pass band of noise is present is believed to be the result of the distortion process and has been studied both physiologically by Deatherage *et al.* (21) and psychophysically by Deatherage *et al.* (20) and by Spieth (96). Attempts to correlate physiological phenomena with behavioral or audiometric measures are on the increase even though it is an area in which conclusions must be made with caution.

#### PHYSIOLOGICAL CORRELATES OF AUDIOMETRIC MEASURES

*Normal threshold.*—The ability of a listener to detect a weak sound depends upon all the physiological elements involved in hearing, from sound transmission through the middle ear to the cortex; any alteration in these pathways may change either the quality of sound or the sensitivity. If the

physiologists studying hearing have one common purpose, it is to find out how each element along the pathways contributes to these two characteristics. The factors that contribute to the over-all sensitivity of the ear certainly are many, and few attempts have been made to analyze the ear completely with this in mind. Wever (108) did a remarkable job in his attempt if we can judge by the fit of his theoretical curve to the actual one. Zwislocki (118) has recently analyzed the transmission characteristics of the ear in order to separate these from the neural factors and has explicitly shown how these vibrating elements contribute to sensitivity. The next way-station is the inner ear from which the cochlear potentials are recorded, and measures of sensitivity at this point, of course, tell us nothing of the effects of the remaining pathways.

There is no real threshold to the cochlear AC potentials; they just appear from the general background of physiological and equipment noise. McGill (70) trained four cats so as to obtain from them a behavioral audiogram for frequencies from 100 to 10,000 cps. After this the animals were tested for sensitivity as determined by cochlear potentials. At sound levels that gave a behavioral threshold, no potentials could be obtained; by extrapolation from a sound pressure that did give threshold, he found the potentials to be extremely low, varying from 0.034 to 0.0023 microvolt. This generally is too low for most amplifiers, but Wever *et al.* (111), using specially constructed equipment, were able to record down to 0.01 microvolt and found the response still decreasing linearly with the sound.

Other comparisons have been made between cochlear potentials and behavioral thresholds. Differences in shape of these curves should show what the nerve pathways contribute to sensitivity. Wever (109) compared the electrical response with conditioned threshold in marmosets, rhesus monkeys, cats, guinea pigs, and pigeons. The differences between curves were remarkably consistent among the different species, showing that sensitivity as determined behaviorally improves more rapidly in the low-tone region than the curve of sensitivity recorded electrically. The electrical curve extends far beyond the behavioral response for high frequencies, and generally the behavioral response shows a much sharper region of maximum sensitivity.

Another way to compare contributions to sensitivity is to overstimulate the ear with either pure tone or noise and compare electrical responses with some behavioral measure of sensitivity.

*Poststimulation thresholds.*—Lawrence, Wolsk & Burton (64) overstimulated a human ear with a pure tone of 1000 cps at 120 db and produced an audiogram with a very sharp loss at 1500 to 2000 cps. Electrical measures from a guinea pig stimulated with the same level and duration of 1000 cps showed a fairly flat loss. This corresponds with other observations that the nervous system somehow sharpens up the end-organ response whether it is normal sensitivity or after injury.

The whole area of stimulation deafness has received considerable interest. It cannot properly be reviewed here. The main emphasis has been in finding

audiometric manifestations of actual physical injury to the organ of Corti, and a whole group of experiments have shown that audiometric shifts in threshold brought about by sounds up to about 80 or 85 db do not reflect end-organ damage but rather some temporary change in neural response. Rahm *et al.* (84) kept a tone of around 60 db in an animal's ear for over 85 hours with no greater loss than 4 db in cochlear potential response. Gisselsson & Sørensen (43) and Sørensen (95) proved a similar point.

Presumably, audiometric measures after high-intensity sound stimulation reflect not only the same neural shift seen following low levels of sound stimulation but also an injury to the organ of Corti. Studies of shifts in cochlear potentials reflect only the organ of Corti injury, and many investigators have continued to use this method to assess the nature of the injury [Lawrence (59); Mizukoshi *et al.* (76); Eldredge & Covell (27); Eldredge, Covell & Gannon (29)]. McCabe & Lawrence (69) and Mangabeira-Albernaz *et al.* (68), using guinea pigs, showed that high-sound levels can destroy the sacculus.

Studies of the recovery process have been made by Eldredge, Covell & Davis (28). Lawrence & Yantis (66) studied the functional recovery and structural repair in overstimulated guinea pigs and found that in a group of equally overstimulated ears, 60 per cent recovered some hearing function, 10 per cent remained the same and 30 per cent showed a further loss. They also showed that Reissner's membrane has the ability to repair itself after rupture.

Minute changes in the cells of the organ of Corti and in the surrounding fluids have shown some subtle changes following overstimulation. Histochemistry, electronmicroscopy, and polarography have been used [Misrahy *et al.* (72); Spoendlin (97, 98); Zorzoli & Boriani (115); Werner (105); and Beck & Michler (2)]. Hammer (46) has made a cytochemical study of the effects upon the ganglion cells.

#### OTHER AREAS

Mention must be made of the attempts to determine the effects of altering blood supply and of blood cooling to the cochlea on the structure of the inner ear as determined by subsequent microscopic examination and electrical recording of the cochlear potentials. Perlman and his co-workers in a series of papers have given a noteworthy description of these changes (37, 51 to 53, 79 to 81). Part of their technique has been to thin out the bone of the otic capsule so as to expose the stria vascularis, and a rather startling observation has been the remarkable stability of vessel size in the stria under various conditions of vascular interruption and hypothermia. It has always been thought that autonomic dysfunction could change the blood supply through the stria and thus give rise to such phenomena as Ménière's disease, but the notion lacks experimental evidence. Other studies of the effect of hypothermia on the cochlear potentials have been carried out by Gulick & Cutt (45) and by Chambers & Lucchina (13).

For many years experiments on the effects of hypoxia upon both structure

and function of the inner ear have been carried out. During the last four years there have been many more: Gulick (44); Fernandez & Alzate (36); Falbe-Hansen *et al.* (32); and Davis *et al.* (19). The effects of oxygen deprivation upon the organ of Corti do not seem to be the same in all experiments. Falbe-Hansen *et al.* did not find the degree of degeneration that Lawrence & Wever (63) found. More experimentation is evidently called for.

Using a delicate micropolarographic technique, Misrahy (73, 74, 75) studied changes in endolymphatic oxygen tension under various conditions; and Wing (112), through studies of the effects upon inner ear function of injections of various substances in the middle ear, discussed the problem of energy-metabolism of the cochlea.

One subject that has been completely omitted from this review concerns the studies of the neural mechanisms of hearing. Research in this area through ablation techniques and microelectrodes has accumulated so rapidly in the last four years that it should take a separate review. Here one could not do it justice, but let it be said in passing that the microelectrode study of hearing is probably throwing more light on brain function than theoretical systems of brain organization ever shed on the mechanism of hearing.

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## COMPARATIVE PHYSIOLOGY: TRANSMITTER SUBSTANCES<sup>1,2</sup>

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The time has passed when it was possible for a reviewer to cover the entire literature concerning his topic. All one can hope for in this age of extensive publication is to give an interpretation of the available data and to guide the reader in his own study by pointing out significant developments and problems. This review does not pretend to be a key to the relevant literature; even a list of all the relevant reviews would exceed the space allocated by the editors for the list of references. Necessarily this review will be an expression of the personal views and opinions of the author since it can only present a selection of the vast amount of literature on the topic, a selection which is guided by what he feels is important and representative of a particular point of view. This review is largely concerned with the research of the past ten years, but whenever this seemed fruitful, earlier literature has been noted.

Since Elliott, in 1904 (1), developed the hypothesis that the cardioaccelerator fibers in the mammal achieve their action on the heart by releasing a local hormone which causes the characteristic response, this hypothesis of neurohumoral action has been well supported and expanded to include vertebrate neuromuscular synapses, synapses between pre- and postganglionic neurons of the autonomic nervous system and central neurons. During the last three decades great progress has been made in the elucidation of synaptic mechanisms in invertebrate animals, and it is possible now to include in a general discussion of the phenomenon of chemical transmission of nerve impulses examples from a large number of animal species ranging throughout the animal kingdom.

The concept of chemical transmission has two aspects: a biophysical, and a biochemical one. At present we find that the biophysically oriented physiologists take chemical transmission almost for granted and base their interpretation of data on this hypothesis. The biochemically oriented physiologists, on the other hand, show more and more skepticism towards evidence concerning the identification of transmitter substances, particularly when this is obtained by pharmacological methods. More and more, the biochemical and biophysical approach are being combined as it becomes evident that only in this way is a satisfactory understanding of the phenomena of synaptic transmission obtainable.

Since its inception, the hypothesis of chemical transmission has been

<sup>1</sup> The survey of literature pertaining to this review was concluded in June 1960.

<sup>2</sup> Among the abbreviations used in this chapter are: ACh (acetylcholine); GABA (gamma-aminobutyric acid); 5-HT (5-hydroxytryptamine).

greatly elaborated and adorned with additional beliefs which have greatly influenced the experimental approach and the interpretation of data. A critical review of the literature reveals, however, that many such beliefs are not supported by sufficient evidence and that the biochemical evidence for specific chemical mechanisms of transmission is surprisingly slim. Such a review shows, however, that in contrast to the popular concepts of chemical transmission, there is now a great amount of information available which necessitates considerable re-evaluation and re-formulation of the original hypothesis and which induces several important new concepts which, if recognized, should greatly influence future research.

#### BIOCHEMICAL EVIDENCE

*Acetylcholine.*—The information on occurrence of acetylcholine (ACh) and cholinesterases in tissues of vertebrate and invertebrate animals has been summarized by Augustinsson (2), by Bacq (3), and by Prosser (4). The vast literature concerned with the actions of ACh on various organs of a great variety of species of several phyla has also been reviewed by Bacq (3), Prosser (4), Krijgsman & Divaris (6), Roeder (7), and by those reviews concerned with synaptic actions in vertebrates. The significance of the information, particularly on invertebrates, can not be established until attempts are made to verify experimentally the assumption that ACh is involved in synaptic transmission in those structures where it occurs and where it has effects.

Acetylcholine has been reported to occur in nervous and nonnervous tissues of species belonging to the phyla *Protozoa*, *Annelida*, *Mollusca*, *Plathelminthes*, *Nemertini*, *Sipunculida*, *Arthropoda*, *Echinodermata*, and *Vertebrata*. Evidence for cholinesterase has been obtained from *Protozoa*, *Coelenterata*, *Nemertini*, *Annelida*, *Plathelminthes*, *Sipunculida*, *Onychophora* (8), *Arthropoda*, *Echinodermata*, and *Vertebrata*.

The information concerning the occurrence of ACh must be accepted with caution. Frequently the compound has been identified solely on the basis of simple bioassays. Several other cholinesters have recently been demonstrated in animal tissues: propionylcholine (9, 10), butyrylcholine (11, 12), imidacyl-acetic acid-cholinester (13), urocanylcholine (14), gamma-amino-butyrylcholine (15, 16),  $\beta$ - $\beta$ -dimethylacrylylcholine, and some unknown ones (17, 18a, b). The biological actions of these compounds may be so similar to those of ACh that, unless chemical identification supplements the bioassays, it is difficult to decide whether an ACh-like action is caused by ACh or by another cholinester. If tested on the frog's rectus abdominis muscle, propionylcholine, imidazolyl-propionylcholine, 2-methylbutyrylcholine, and butyrylcholine are 4.5, 3.0, 1.6, 1.38, and 1.15 times as active as ACh. Carbaminoylcholine, on the rabbit intestine, and butyrylcholine, on the leech muscle, are nearly as effective as ACh (17, 18a, b).

That the occurrence of ACh in a tissue must not be taken as indication that it is present in nerve cells can be seen from the finding of the compound

and cholinacetylase in nerve-free tissue such as human placenta [Hagen (19)] and spleen [Dale & Dudley (20)]. Bülbring, Burn & Shelley (21) emphasized this fact in discussing their finding of ACh, cholinesterase, and cholinacetylase [see also Milton (22)] in the gills of the mussel *Mytilus edulis*, assuming this structure to be nerve free. The latter assumption, however, is unfounded since lamellibranch gills are known to be innervated by fibers originating in the visceral ganglion.

The finding of cholinesterase activity does not necessarily imply ACh as transmitter, as this rather common enzyme system may well be involved in functions other than ACh-hydrolysis (see below).

The presence of the ACh-synthesizing enzyme cholinacetylase has been demonstrated in the following structures of invertebrates: heads of blowfly, *Calliphora* [Smallman (23)], housefly, *Musca* [Frontali (10)], squid ganglia [Nachmansohn & Weiss (24)], and *Mytilus* gills (21). Acetylcholine definitely has been identified in heads of bees [Augustinsson & Grahn (17)], houseflies [Lewis (25)], and blowflies [Chefurka & Smallman (26)]. The identity of chemically isolated ACh from *Octopus* ganglia [Bacq & Mazza (27)] is rather certain.

Among the more than one million species of invertebrates, transmission of nerve action by ACh has been demonstrated only in three cases on the basis of the following evidence: release of an ACh-like substance into perfusion medium during stimulation of presynaptic neurons; imitation of nerve action by artificially applied ACh; potentiation of nerve action and of ACh by application of anticholinesterases; blocking of nerve action and of ACh by the same blocking agent (curare, atropine, etc.). The three cases are: neuromuscular junctions of longitudinal muscles of *Hirudo medicinalis* [Bacq & Coppee (28, 29)], neuromuscular junctions of retractor muscles of the holothurian *Stichopus regalis* [Bacq (30)], and synapses between cardio-accelerator fibers and heart muscle of the clam *Venus mercenaria* [Prosser (31), Welsh (32)]. During the past two decades great attention has been paid to the possibility of cholinergic mechanisms in mollusks and arthropods. The heart of cephalopods and of many lamellibranchs is inhibited by ACh and by stimulation of the visceral ganglion or the cardioregulator nerves. In the lamellibranch *Venus mercenaria*, ACh not only imitates the action of the cardioinhibitory nerves (31, 32), but eserine potentiates (31) and mytolon blocks (32) both the action of the inhibitory nerves and that of ACh. It is not known whether the inhibitory nerve acts directly on the heart muscle or whether it acts on ganglion cells of the auricles. So far, no ganglion cells have been described for the *Venus* heart (31) and it is uncertain whether or not the inhibitory nerves supply the ventricular muscle as well as the auricles. Since isolated ventricles of *Venus* and of other lamellibranchs are inhibited by low concentrations of ACh, it is impossible to come to a conclusion about the normal role or origin of ACh in cardiac inhibition. It is conceivable that the cardiac nerves cause the release of ACh from cardiac ganglion cells and that they themselves are noncholinergic.

In view of the finding of ganglion cells in the heart of many lamellibranchs [Dogiel (34); Suzuki (35, 36)], gastropods [Suzuki (37); Morin & Jullien (38)], and cephalopods [Ransom (39)], it is not possible to claim a myogenic origin of the heartbeat [Krijgsman & Divaris (6)]; and the opinion that myogenic hearts are inhibited by ACh while neurogenic hearts are accelerated [Prosser (4, 40); Krijgsman & Divaris (6)] is premature. It should be pointed out that the action of ACh on vertebrate and invertebrate hearts is not always inhibitory. In ventricles of the clams *Mytilus galloprovincialis*, *M. californianus*, *Amphidesma forsterianum*, and *A. ventricosum*, ACh causes prolonged systolic contraction [Jullien & Vincent (41); Pilgrim (42); Florey & Merwin (43)]; and in the lamprey *Lapetra fluviatilis*, this substance causes pronounced cardioacceleration [Augustinsson, Faenge, Johnels & Oestlund (44)]. (On the heart of *Lapetra*, epinephrine, norepinephrine, and 5-hydroxytryptamine have only weak effects.) The obviously neurogenic hearts of decapod crustacea and xiphosura are accelerated by ACh [Welsh (45, 46); Garrey (48)].

In spite of the uncertainty of the normal role of ACh in cardiac control of the mollusc heart, this organ, prepared from *Venus mercenaria*, has yielded much valuable information in the hands of Welsh & Taub (49 to 52), concerning structure-activity relationship of ACh and related compounds. The findings lend important support to the assumption that postsynaptic membranes are equipped with receptor molecules of specific structure which react with the transmitter substance to give rise to the characteristic postsynaptic reaction.

For a time it seemed reasonably certain that the heart of decapod crustacea is accelerated by cholinergic acceleratory nerves. Welsh (45 to 47) had reported that ACh causes marked acceleration of the heart of lobsters and crabs and that the nervous system of these animals contains considerable amounts of ACh. These findings have been confirmed and extended by several workers [Prosser (40); Davenport (53, 54); Smith (55); Easton (56); Florey (57), etc.]. Extracts of central nervous system of decapods were found by Welsh (46) to accelerate the heart in a manner similar to that of ACh or cardioaccelerator stimulation. The similarity between the action of cardioaccelerators and ACh was further demonstrated by Wiersma & Novitski (58) in the crayfish. These authors reported a slight potentiation of cardioaccelerator action by eserine while others failed to observe this phenomenon in *Cancer irroratus* (59), *Pacifastacus leniusculus*, *Orconectes virilis*, and *Cancer magister* (60). It appears now (60) that ACh is not normally involved in cardioacceleration in the decapod crustacea for the following reasons: the cardioaccelerator fibers and the cardiac ganglion of the lobster *Homarus americanus* were found to contain no detectable amount of ACh (less than 0.1  $\mu\text{g./gm.}$ ). Atropine, which effectively blocks the action of ACh on the heart (ganglion), does not alter the response to stimulation of the cardioacceleratory fibers; eserine, which potentiates the action of ACh on the heart, does not alter the response to accelerator stimulation. It should be mentioned that the stimulating effect of crustacean nerve extract on the heart is

not attributable to ACh because the effect is not prevented by atropine [Florey & Florey (61)].

Acetylcholine has no effect on crustacean somatic muscle [Bacq (62); Katz (63); Ellis, Thienes & Wiersma (64)]. Its presence in peripheral nerve is explained by its occurrence (probably exclusively) in the sensory fibers [Florey & Biederman (65)]. Transmission of nerve impulses from sensory fibers by way of ACh has not been established. It is interesting that stretch receptor neurons of crayfish and lobster are stimulated by low concentrations of ACh [Wiersma, Furshpan & Florey (66)]. It is likely that the rapid contractions of muscles in isolated crustacean limbs after injection of ACh (61) and the autotomy of limbs after ACh injection [Welsh & Haskin (67)] are caused by stimulation of sensory neurons in the injected legs and by ephaptic or reflex transmission of this excitation to motor-axons [Florey (68)].

The evidence for cholinergic transmission in the vertebrates has been ably reviewed by Rosenblueth (69), Hebb (70), and others. A cholinergic mechanism has been definitely established for the mammalian and amphibian motoneurons. The originally rather straightforward picture of cholinergic pre-ganglionic sympathetic and parasympathetic neurons and of postganglionic parasympathetic neurons has recently been complicated by several developments. On the basis of histological studies [see Boeke (71)], Meyling (72), Jabonero (73), and others proposed that the peripheral autonomic nervous system is not only composed of pre- and post-ganglionic neurons, but of a third element, an adrenergic groundplexus of syncytially connected nerve cells which is supposed to mediate the actions of both postganglionic sympathetic and parasympathetic neurons. Indeed, Middleton and co-workers (74, 75) described an atropine-resistant cardioacceleration produced by vagal stimulation and concomitant release of an epinephrine-like agent. Koshtoiants & Putintseva (76, 77) described the restorative action of vagal stimulation during potassium-iodide-induced cardiac arrest, and cardioacceleration during vagal stimulation in frog hearts recovering from potassium iodide treatment. The action was confined to the right vagus and was unaffected by atropine but abolished by adrenergic blocking agents and by nicotine. All these experiments were interpreted under the assumption of epinephrine-releasing cells (neurons or chromaffin cells in the heart muscle). Koshtoiants & Putintseva believed in the report of Kibiakov & Tukhvatullina (78) that there are no sympathetic fibers present in the vagus nerve of *Rana temporaria*. It is interesting that Otto Loewi's classical demonstration (79) of humoral transmission by *Acceleransstoff* was carried out by stimulating the vagus nerve supplying the atropinized heart of *Rana temporaria*. Loewi also showed that in toads it is not even necessary to resort to atropine since, during the summer, stimulation of the vagus always causes acceleration. He refers to the "well-known fact" that the vagus contains sympathetic fibers. This has now been confirmed for the cat by Benitez, Holmgren & Middleton (80) who therewith explain the previous results of Middleton *et al.*

Hillarp (81) has now critically examined the histological evidence and, on

the basis of new studies, comes to the conclusion that the terminal groundplexus consists of a terminal reticulum formed of the endings of the postganglionic parasympathetic and sympathetic neurons. He clearly shows the interstitial cells to be nonnervous.

The apparently so well established fact of cholinergic transmission in sympathetic ganglia of mammals has recently been questioned by Kewitz & Reinert (82 to 85). These authors have shown that epinephrine and norepinephrine enhance the action of ACh but depress transmission, while atropine, scopolamine, amphetamine, *p*-hydroxyphenylethanolamine, and methamphetamine after an initial excitation inhibit the ACh action without disturbing transmission. Furthermore it was found that surface active anesthetics like pantocaine and procaineamide inhibit synaptic transmission in sympathetic ganglia without altering the ACh sensitivity of the postganglionic cells to ACh. Similarly, it had been shown that the action of the vagus of the stomach is not blocked by doses of atropine which completely prevent the action of applied ACh. Greef & Holtz (86) have, however, shown that during vagus action on the stomach a substance is released into the perfusion fluid which stimulates the isolated ileum and is antagonized by atropine and which Greef & Holtz assume to be ACh. A new approach to the problem is suggested by Fehér & Bokri (87) who report findings that are principally similar to those of Kewitz. They assume that there are two types of ACh receptors in the postsynaptic membrane: free receptors, not normally involved in transmission, and "innervated" receptors which react with the released transmitter. The former are highly sensitive to applied ACh and are inhibited by atropine, succinylcholine, choline, and cholinephenylether, and the latter are relatively insensitive to applied ACh and are inhibited by deka- and pentamethonium.

Acetylcholine may well have actions within tissue cells. Its function within heart muscle cells of vertebrates has recently been discussed by Burn (88) and Rothscluh (89). In this connection it is interesting that according to Koeber and co-workers (90, 91, 92) about 50 per cent of the cholinesterase activity of rabbit and dog striated muscle is found in the myosin and mero-myosin fractions.

*Epinephrine, norepinephrine, catecholamines.*—Reviews concerning occurrence and action of epinephrine and norepinephrine in vertebrate animals have been published in recent years, notably the books by von Euler (93) and Rosenblueth (69) [see also (4, 6, 94, 95)]. The metabolism of epinephrine and other sympathicomimetic amines has been discussed recently by Bacq (96) and by Axelrod (97). It is now rather well established that norepinephrine is the principal neurohumor of adrenergic neurons and that the enzyme which normally inactivates the catecholamines in mammals is not aminoxidase but *o*-methyltransferase [Axelrod & Tomchick (98); see also (99)].

The adrenergic nature of postganglionic sympathetic neurons has lately been further substantiated by the demonstration of oxityramine (dopamine), the precursor of norepinephrine, and dopadecarboxylase in postganglionic

sympathetic nerves of cattle by Holtz & Westermann (100) and by Schue-mann (101). Goodall & Kirshner (102) have shown that sympathetic nerves and ganglia, but not the vagus nerve, are capable of synthesizing hydroxy-tyramine and norepinephrine from dihydroxyphenylalanine.

Evidence for adrenergic synapses in any invertebrate species is virtually non-existent, although epinephrine, norepinephrine, and dopamine have been identified in insects *Tenebrio* and *Apis* [Wense (103); Oestlund (104)] and epinephrine in annelids [Wense (103)]. Von Euler (105) found indications for the presence of norepinephrine in gill hearts and gills of *Octopus vulgaris* (0.04 and 0.015  $\mu\text{g./gm.}$  respectively). Rather large amounts of norepinephrine occur in the posterior salivary gland of *Octopus vulgaris* (10  $\mu\text{g./gm.}$ ) and smaller amounts (1  $\mu\text{g./gm.}$ ) in those of *Octopus macropus*. No traces of epinephrine were found in these molluscs. No one has ever described the occurrence of epinephrine or norepinephrine in any invertebrate species outside the annelids, insects, and cephalopods. Oestlund (104), as well as von Euler (105), was unable to detect these compounds in several species of crustacea, and von Euler stated that extracts of whole echinoderms (*Echinus*) contained less than 0.01  $\mu\text{g./gm.}$  (dry weight) of norepinephrine-like activity. According to Bacq (3), epinephrine does not occur in sponges and coelenterates.

It is of interest that epinephrine, as well as norepinephrine, has been found to have a powerful action on a number of organs in invertebrates [for literature until 1950 see the compilation in Prosser (4)]. The hearts of cephalopods are accelerated by epinephrine as well as norepinephrine [Erspamer & Ghiretti (106); Faenge & Oestlund (107); Florey & Florey (61)] and epinephrine has been found to accelerate the heart beat of several crustacea [*Daphnia*, Florey (57); *Dromia*, *Calappa*, *Eriphia*, *Carcinus*, *Palinurus*, Florey & Florey (61); and insects [*Corethra*, Florey (57)].

Epinephrine was found to be without effect on the hearts of certain mollusks [*Helix*, Boyer (108), Erspamer & Ghiretti (106); *Aplysia*, von Euler *et al.* (109); *Anodonta*, Faenge (110)] and fish [*Myxine*, Faenge & Oestlund (107)].

Epinephrine and norepinephrine have a stimulating action on the isolated intestine of the crayfish [Florey (111)]. Both compounds are very effective in causing the dispersion of pigment in the dark chromatophores of the shrimp *Crangon vulgaris* and of the isopod *Idothea tricuspidata* [Pautsch (112); Florey (113)], and they cause pigment concentration in the isopod *Mesidothea entomon* [Pautsch (112)]. The actions of epinephrine and norepinephrine in crustacea are particularly striking as these compounds apparently do not occur in these animals (104).

*5-Hydroxytryptamine (5-HT).*—There is suggestive evidence that 5-HT acts as a transmitter substance in certain animals. This is largely obtained from work with molluscs; 5-HT occurs in the nervous system of cephalopods, gastropods, and lamellibranchs [for a compilation of the data see the review of Erspamer (114, 115); Page (116); Welsh (117, 118)], and mono-aminoxi-



dase, the inactivating enzyme, has been detected in many molluscan organs [Blaschko & Himms (119); Blaschko & Hope (120)]. In very low concentrations, 5-HT mimics the action of cardioaccelerator nerves. Obviously the evidence for a transmitter function of 5-HT in molluscs is not conclusive, because: (a) 5-HT has never been demonstrated in nerve fibers which supply molluscan organs; (b) the release of 5-HT from stimulated nerve fibers (or ganglia) has not been demonstrated; (c) agents [e.g., LSD according to Welsh (121)] which block the action of 5-HT in certain mollusc hearts have never been shown to prevent the action of cardioaccelerator fibers.

Brodie & Shore (122) suggested that 5-HT is the transmitter substance of the central parasympathetic system in mammals. The merits of this hypothesis and its critique have been discussed by Page (116). The suggestion has been made by Marazzi and co-workers [see Marazzi (123)] that 5-HT is a transmitter substance of certain inhibitory neurons within the brain of mammals, because it was found that this substance imitates natural synaptic inhibition and that similar inhibition can be brought about by application of the amino-oxidase inhibitor iproniazid [Gluckman, Hart & Marazzi (124)]. The evidence is, however, still inconclusive because it has not been shown that the inhibitory pathway contains 5-HT, or that chlorpromazine, which blocks the 5-HT action, also prevents normal synaptic inhibition. The inhibition by iproniazid can also be interpreted as the result of a direct action on the postsynaptic membrane, particularly as this drug does not potentiate the action of presynaptic neurons but imitates it. A good deal of literature has been published which favors a role of 5-HT in normal brain function and implies an abnormal 5-HT metabolism as the cause of mental disturbance and disease. No reference will be made to this literature, since it contains no data specific enough to support the assumption that 5-HT might be a transmitter substance in the mammalian central nervous system.

Compared to the quantities of 5-HT found in other tissues, the amounts found in mammalian brain are negligible although their distribution is restricted to certain brain structures, particularly the hypothalamus and mesencephalon [0.2 to 0.33  $\mu\text{g./gm.}$  fresh tissue (125)]. The largest amounts of 5-HT ever discovered in animal tissue occur in human metastatic carcinoids of the small intestine: 2500  $\mu\text{g./gm.}$ , according to Lembeck (126); 2300  $\mu\text{g./gm.}$  dry weight in ox blood platelets were found by Zucker, Friedman & Rapport (127). Large quantities of 5-HT are present in the posterior salivary glands of certain cephalopods [760  $\mu\text{g./gm.}$  in *Eledone moschata* (114)], in the hypobranchial gland of the marine snail *Murex trunculus* [290  $\mu\text{g./gm.}$  (114)], in the venom of *Vespa vulgaris* [320  $\mu\text{g./gm.}$  (128)], the skin of the toad *Bombinator pachypus* [1000  $\mu\text{g./gm.}$  (114)], and in coelenteric tissue of the sea anemone *Caliactis parasitica* [600  $\mu\text{g./gm.}$  dry wt. (129)]. The occurrence of 5-HT in the nervous system (ganglia) of several molluscs (including gastropods, lamellibranchs, and cephalopods), as determined by bioassay methods [Florey & Florey (61); Welsh (32)], has been recently confirmed with the use of a spectrofluorometric method; the values range from

2 to 50  $\mu\text{g./gm.}$  [Welsh & Moorhead (130)]. The finding (61) that the central and peripheral nervous system of decapod crustacea contains a substance with biological activities similar to those of 5-HT has been supplemented by later reports that 5-HT does not occur in the crustacean nervous system but that a similar compound, an orthodihydroxytryptamine [Carlisle (131)], is responsible for the actions. This compound appears to be the "neurosecretion" of the pericardial organs [Alexandrowicz (132)] which causes cardioacceleration in decapod crustacea, with the exception of *Maja squinado*, the spider crab, in which animal the same substance (and 5-HT) produces cardiac arrest (133). Ross (134) has described responses to 5-HT (contraction, facilitation) of muscles of sea anemones. 5-HT was found to stimulate various sensory receptors in mammals (135 to 137) and crayfish (68).

*Substance P.*—This substance has been defined by Pernow (138) as a polypeptide. It stimulates vertebrate smooth muscle preparations and occurs not only in the intestine but also in the central and peripheral nervous system. Several papers report on the occurrence and distribution of this substance in the nervous system of mammals, birds, reptiles, and amphibia (138 to 142). The substance has never been described in animals other than vertebrates. Lembeck (142) finds about 10 times as much in dorsal as in ventral spinal roots of cattle. In conjunction with his finding that chromatographically purified Substance P causes vasodilation in rabbits ears in a manner similar to that brought about by antidromic stimulation of the (sensory) auricular nerve, he concludes that Substance P might well be the long-sought-for sensory transmitter agent (see below). His suggestion was supported by Andrews & Holton (143) who found that during degeneration of the auricular nerve its content of Substance P falls (in contrast to that of adenosine triphosphate which remains constant). Umrath (144), however, is of the opinion that the Substance P of sensory nerves is the precursor of the sensory transmitter. He finds that an enzyme from the spinal cord can rapidly inactivate the smooth-muscle-stimulating property of Substance P obtained from sensory nerves, until only a residual activity is left which remains constant. This remaining activity is about equal to that originally present in fresh ventral root extracts (145). The latter activity is not attacked by the enzyme. Umrath (144) also reports that during degeneration of the auricular nerve in the rabbit, the Substance P content falls during the first two days to 60 per cent and then remains constant during the next 13 days. The vasodilator substance, which according to Hellauer & Umrath (146) (see below) represents the sensory transmitter, drops during the first three days to about 50 per cent and remains constant to the sixth day when it diminishes rapidly just when the Substance P-inactivating enzyme begins to disappear. Substance P, on inactivation, gives rise to the vasodilator substance. Umrath (145) believes that the Substance P of motor neurons contains, before its extraction, ACh as a side chain and that this molecule represents the precursor of ACh. The suggestion is supported by circumstantial evidence only.

On the basis of his findings that Substance P has centrally depressant actions and that it antagonizes strychnine- and picrotoxin-induced convulsions in mice, Zetler (147) suggests that Substance P might be an inhibitory transmitter substance.

The speculations connected with Substance P are indeed intriguing but it must be admitted that the supporting evidence is not yet satisfactory.

*Sensory transmitter, adenosine triphosphate.*—Loewi & Hellauer (148) had already found that dorsal spinal roots (cattle) contain almost no ACh. According to Wolfgram (149), the dorsal roots (horse) contain an extremely small amount of cholinacetylase, in contrast to the large amounts in the ACh-containing ventral roots. It appears that sensory fibers of mammals are noncholinergic. In an attempt to find the transmitter of these fibers, Hellauer & Umrath (146, 150) followed a suggestion of Dale (151) that a neuron releases the same transmitter at all its endings. Since the capillary vasodilator fibers in the mammalian skin are supposed to be sensory collaterals, these authors used dilation of skin capillaries as indication for the presence of the "sensory substance". They found that sensory fibers do indeed contain a vasodilator substance to which the capillaries become hypersensitive after sensory denervation. Hellauer (152) has determined some of the chemical properties of this agent. Perhaps the most important finding is that the apparent enzymatic inactivation of the substance during incubation with nerve tissue homogenates is prevented by the convulsant agents strychnine, picrotoxin, and pentylenetetrazol in the same relative concentrations in which these drugs induce convulsions (150). The work of Umrath & Hellauer was challenged by Holton and co-workers (154 to 157). These authors showed that the sensory nerve endings at the capillaries in the rabbit's ear release adenosine triphosphate upon stimulation and that this substance causes vasodilatation. This agent occurs, however, in dorsal as well as ventral spinal roots and for this reason alone cannot be the agent studied by Hellauer & Umrath. Furthermore, it is not inactivated by enzymes that can be inhibited by strychnine and picrotoxin. Florey & McLennan (158) have confirmed that the vasodilation caused by dorsal root extracts is very much stronger than that caused by ventral root extracts in denervated rabbit ears. In contrast to the response to dorsal root extract, there is no potentiation of the adenosine triphosphate action after denervation.

On the assumption that convulsant agents exert their action by inhibiting enzymes which normally inactivate sensory transmitters, Florey (159) investigated the actions of strychnine, picrotoxin, and pentylenetetrazol (Metrazol) in a large number of species ranging throughout the animal kingdom. The results indicated an astonishing parallel between taxonomic position and responsiveness to the convulsant action of the drugs; all species belonging to the group of *Deuterostomia*, whether they were mammals, fish, tunicates, chaethognats, or echinoderms, responded to strychnine, picrotoxin, and pentylenetetrazol with reactions equivalent to convulsions. In

these cases the effective minimal doses of the drugs were related as 1:5:40. All arthropods, including *Insecta*, *Arachnoidea*, *Malacostraca*, *Cirripedia*, and *Phyllopoda*, were found to respond to amazingly low concentrations of picrotoxin but to be refractory to strychnine. The annelids (*Oligochaeta* as well as *Polychaeta*) show no response to picrotoxin but become excited under the influence of strychnine. *Platyhelminthes* and *Nemertini* behave like *Deuterostomia*: strychnine, picrotoxin, and pentylenetetrazol elicit convulsive movements; the minimal effective doses are related as 1:5:40. Among the molluscs it was found that the *Cephalopoda* exhibited an extraordinary sensitivity to strychnine [see also Baglioni (160); Froehlich (161)] while they were insensitive to picrotoxin. A similar behavior was found with the *Opisthobranchia*. The *Pulmonata* are insensitive to strychnine, pentylenetetrazol, and picrotoxin. No specific reactions could be elicited with any of the drugs in the coelenterates. Umrath (162) and Egghart & Umrath (163) have expanded the investigation and have led the argument *ad absurdum* that the drug-induced convulsions arise from inhibition by the drugs of the enzymes which destroy the sensory transmitter. Not only is the interpretation of the reactions of whole animals treated with drugs always difficult, but it is impossible to ascribe these to simple causes. Umrath concluded, for instance, that the "sensory enzyme" of the annelids is inhibited by anticholinesterases and that therefore the sensory transmitter must be chemically related to ACh. The evidence on which this hypothesis is based is as follows. The minimal doses of a number of convulsant agents are related to but not equal to their anticholinesterase activity. Acetylcholine, as well as drugs which have anticholinesterase activity, induces contractions and convulsive movements. Much of the argument for the action of convulsant drugs on the "sensory enzyme" became uncertain when Bradley, Easton & Eccles (164) proved that strychnine blocked the action of the inhibitory neurons in the mammalian spinal cord. It has since been possible to explain the convulsant action of picrotoxin in crustacea on the same basis [Elliott & Florey (165)]. In spite of the striking sensitizing actions of the convulsant drugs, their actions can no longer be taken as evidence for inhibition of enzymes which inactivate the sensory transmitter. Nevertheless, the experiments of Umrath & Hellauer which show inhibition by convulsant agents of enzymatic inactivation of the vasodilator substance of mammalian sensory nerves must be kept in mind.

The only experiments which used living nerve tissue as an indicator for the hypothetical sensory transmitter were those which used the optomotoric reactions of bees [Florey (159)]. With a simple operation it is possible to introduce nerve extracts and drugs into the first optic ganglion where the first synapses between primary and secondary sensory neurons are located. Introduction of dorsal root extracts (cattle) or of ACh-free extract of crayfish central nervous system into one eye causes circus movement towards the treated side, while low concentrations of ACh and ventral-root extracts (cattle) induce movement in the opposite direction. With negative phototactic bees the reactions are reversed, indicating that the active agents

enhance or diminish the "sensation" of light. Picrotoxin and eserine elicit circus movements in the same sense as sensory nerve extracts and ACh, respectively, but they have this action only while the eyes are illuminated, indicating that they may well interact with transmitter substances produced by photoreceptors in response to light. The action of picrotoxin was originally interpreted in the light of Umrath & Hellauer's findings, but the recent demonstration of the blocking of Factor I action by picrotoxin offers an alternative explanation, particularly since inhibitory interactions in the retinula of *Limulus* are now well known [see (166)]. Picrotoxin, therefore, might achieve its action not by preserving the sensory transmitter but by preventing mutual inhibition of sensory elements.

*Inhibitory transmitter, Factor I and Substance I.*—Although it was held possible for a long time that one and the same nerve cell can have excitatory as well as inhibitory actions, there is now overwhelming evidence for the existence of specific inhibitory neurons. With the exception of the inhibitory fibers of the vertebrate autonomic nervous system which are either cholinergic or adrenergic, there is good reason to believe that the inhibitory neurons are characterized by a substance, or complex of substances, referred to as Factor I [Florey (167)]. The characteristic distribution of Factor I in the mammalian brain [Florey & Florey (168)] indicates the presence in certain nuclei and fiber tracts of "I-neurons". The existence of such I-neurons has been confirmed in crabs where it was found that Factor I occurs exclusively in the inhibitory neurons and is absent from motor and sensory neurons (65). Factor I has originally been defined as that agent in mammalian brain extracts which is responsible for the reversible inhibition of impulse generation in the slow-adapting neuron of the abdominal stretch receptor organs of crayfish (167). Since then Factor I has been purified [Florey & McLennan (170, 171); McLennan (173)] and applied to a variety of vertebrate and invertebrate test-preparations (167 to 172; 174 to 176). The following actions were ascribed to Factor I: inhibition of crustacean stretch receptors, inhibition of crustacean heart ganglia, inhibition of spontaneous contractions of the isolated hindgut of crayfish, inhibition of ACh-induced and spontaneous contractions of the isolated hindgut of crayfish and of the ileum of rabbit and guinea pig, inhibition of monosynaptic reflexes in cats, blockade of synaptic transmission in autonomic ganglia of cat and rabbit (exception: superior cervical ganglion), prevention of strychnine- and picrotoxin-induced convulsions in mice, inhibition of heartbeat in octopus, stimulation of rectum of octopus and squid, and inhibition of ACh-induced contractions of sea urchin esophagus.

Although Bazemore, Elliott & Florey (177) identified Factor I with gamma-aminobutyric acid (GABA), it soon became evident that this substance could not duplicate most of the actions ascribed to Factor I preparations, although it conformed to the original definition of Factor I inasmuch as it inhibited impulse generation in the slow-adapting sensory neuron of

crayfish stretch receptors. McLennan (178) has shown that Factor I preparations prepared according to Florey & McLennan may contain no gamma-aminobutyric acid, and he described two active fractions of Factor I which he has chromatographically isolated and which he calls fractions A and B. He believed fraction B to be a guanidino compound, and now assumes that fraction A is the ionized form of fraction B (173). Fractions A and B have identical actions; they not only inhibit crayfish stretch receptors but also block monosynaptic reflexes in the cat and transmission in autonomic ganglia. Their action on the spinal cord is prevented by strychnine, and the action on the stretch receptors (like that of GABA!) is blocked by picrotoxin.

Gamma-aminobutyric acid appears to be absent in extracts of central and peripheral nerve tissue of lobster and crab [Florey & Chapman (179)]. There occurs, however, a single inhibitory substance which has been chromatographically isolated. It was named Substance I [Florey (180)]. Substance I appears to be identical with fraction A of Factor I.

There is sufficient evidence that GABA is not the inhibitory transmitter although it imitates the transmitter action in many crustacean preparations (181, 182). There is, however, good evidence that Substance I is the transmitter substance of inhibitory neurons in crustacea, and it is quite possible that it is the transmitter in vertebrates as well. The following can be quoted. (a) Substance I imitates the action of inhibitory neurons inasmuch as it inhibits stretch receptors, cardiac ganglion cells, and muscular contraction in decapod crustacea. (b) Its action, like that of inhibitory fibers, is prevented by picrotoxin. (c) It occurs exclusively in inhibitory neurons. (d) On stimulation of inhibitory neurons, a substance is released into the perfusion fluid which acts like the inhibitory fiber and whose action is blocked by picrotoxin. (e) According to McLennan (173), fraction A of Factor I inhibits monosynaptic spinal reflexes in the cat by causing hyperpolarization of motoneurons. (f) This action is blocked by strychnine as is the action of the inhibitory fibers involved in this reflex pathway. (g) Factor I can be extracted from the spinal cord and brain of mammals but not from peripheral and autonomic nerves and ganglia which contain no inhibitory neurons.

That the active component of Factor I can occur in an ionized and non-ionized form may well explain the repeatedly made observation that Factor I extracts are more active at pH values below 7.0 (167, 170). McLennan finds the specific activity of fraction A to be about 6 times higher than that of the un-ionized fraction B. A shift from A to B may well be all that is required to inactivate the inhibitory transmitter physiologically.

The occurrence and pharmacological actions of GABA have been the subject of recent reviews (183 to 185), and in 1959 a symposium was devoted to its biochemistry and physiological actions (186).

Inhibitory agents from mammalian brain have been described by Pfeiffer & Pataky (187, 188) and Lissak & Endroeczi (189, 190). They have certain actions in addition to those reported for Factor I. This may well be because

they have been applied to some test-organs in extraordinarily high doses. It is very likely that these unidentified agents are identical with the active agents of Factor (and Substance) I.

The significance of the inhibitory and depressant effects of topically or intraventricularly applied gamma-aminobutyrylcholine and gamma-aminobeta-hydroxybutyric acid on the motor cortex of mammals [Takahashi *et al.* (191); Hayashi (192)] can only be evaluated when attempts are made to establish the role of these agents as transmitters.

#### THEORETICAL CONSIDERATIONS

The widely accepted concept of chemical transmission can be formulated as follows. (a) Although it may be concentrated in the nerve endings, the transmitter (or its immediate precursor) occurs everywhere in the neuron. (b) Axon and nerve endings contain the enzyme system capable of synthesizing the transmitter. (c) The transmitter is stored in a physiologically inactive, bound form. (d) The transmitter is released by the nerve endings upon the arrival of a nerve impulse. The amount released is rather constant. (e) The free transmitter diffuses across the synaptic gap. (f) It reacts with specific receptor molecules located in the subsynaptic membrane, giving rise to the postsynaptic response. (g) An enzyme system located in the synaptic region inactivates the transmitter. Such enzymatic inactivation, however, is not absolutely essential since diffusion alone may effectively remove the transmitter from its original point of action. (h) Only the subsynaptic patches of the postsynaptic membrane are specifically sensitive to the transmitter agent, but after denervation the sensitive areas spread over the whole surface of the postsynaptic cell.

Several additional beliefs can be found in the literature:

- (a) One nerve ending releases only one transmitter agent.
- (b) A nerve fiber releases the same transmitter at all its endings [called "Dale's principle" by Eccles (193)].
- (c) In spite of the great morphological divergence, there is only a very small number of different transmitters.
- (d) Cholinergic neurons alternate with noncholinergic neurons.
- (e) Transmitters can act only on those structures which are normally innervated by nerve fibers which release this transmitter.

Before dealing with the general concept of chemical transmission, it appears worthwhile to discuss these "beliefs" and to examine their validity.

(a) There is no conclusive proof that nerve endings cannot and do not release more than one transmitter substance, but there is some evidence that nerve fibers contain two substances which have proven transmitter function in other structures. Postganglionic sympathetic nerves contain not only epinephrine but also ACh. Although it has not been shown that these compounds occur in the same neurons, the possibility definitely exists. Mammalian



sensory fibers contain two vasodilator principles; one of them, adenosine triphosphate, is released upon stimulation—the other might well be a vasodilator-transmitter (157). Crustacean sensory fibers contain ACh as well as another excitatory substance; both could be transmitter substances but the transmitter function has not been established for either of them (169).

(b) The only proof for Dale's principle is the finding that the central collaterals of lower motoneurons of the cat are cholinergic as evident from the facts that their action is blocked by di-hydro-beta-erythroidin and prolonged by anticholinesterases and that ACh imitates their action (194). It would be most important to prove the validity of the principle in the case of the inhibitory fibers of crayfish which have synaptic endings on dendrites (and cellbody?) of stretch receptor neurons and on muscle fibers.

(c) Although the almost universal occurrence of cholinesterases is well established, the general role of ACh as a transmitter agent is not too well documented. In the past years several cholinesters have been identified in nerve tissue (see p. 502), and many findings of ACh-like activity with the aid of bioassays are open to question wherever the chemical nature of the responsible agent has not been definitely established. For mammalian pre-ganglionic sympathetic and pre- and postganglionic parasympathetic neurons and for motor-fibers, the acetylcholinergic nature is generally accepted. But these are only three well-defined types of neurons. We are by no means sure that all motor fibers are cholinergic, and there is as yet little evidence that the ACh of the vertebrate central nervous system belongs to many different types of nerve cells. Factor I action and the mechanism of action of strychnine in the mammalian nervous system have only been shown in synapses of monosynaptic spinal reflexes, and Substance I was isolated from peripheral inhibitory fibers that supply the leg muscles of crabs. For the time being, the assumption of a small number of different transmitter substances is not based on evidence but rather on the admittedly strong argument that not many compounds could qualify as transmitter agents: (a) out of thousands of compounds only a few have strong pharmacological actions; (b) of these only a few occur in nerve tissue; and (c) many neurons innervating quite different effectors are all cholinergic. In addition, it seems reasonable to assume that cells derived from the same "mother cell" during ontogeny retain the same neurochemical pattern.

(d) On the basis of their studies on the distribution of cholinacetylase in dog brains, Feldberg & Vogt (195) concluded that there is a general pattern of alternating cholinergic and noncholinergic neurons. This scheme has sometimes been interpreted as implying that cholinergic neurons are not cholinceptive. The concept, of course, is disproved by the generally accepted assumption that pre- and postganglionic parasympathetic neurons are cholinergic. Furthermore, those regions of the brain which contain the mass of cholinergic neurons (as evidenced by the large amounts of cholinacetylase activity) also contain other biologically active substances, such as 5-HT,

Substance P, sympathin, and Factor I (125, 139, 140, 168), indicative of noncholinergic neurons. Acetylcholine-containing nerve cells can certainly respond to ACh as the crustacean stretch receptor neurons show.

(e) Edwards & Kuffler (181, 182) recently stated that gamma-aminobutyric acid cannot be the transmitter substance of crustacean inhibitory neurons because it also inhibits nerve cells which receive no inhibitory innervation. The absence of nerve fibers is always difficult to prove; and there is good evidence, particularly for sensory neurons, that nerve cells respond to transmitter agents which do not normally act on them. Acetylcholine has been shown to stimulate not only crustacean stretch receptors, but almost all mechanoreceptors of vertebrates [see the important critical review by Witzleb (196)]. Since those drugs which block this action of ACh do not interfere with the excitation of the nerve fibers that is brought about by the adequate stimulation, it must be assumed that these cells are not normally excited by ACh. Since such cells respond to anticholinesterases and ACh-blocking agents in the usual manner, one must conclude (a) that they possess cholinceptive receptors and (b) that cholinceptive membranes are a very common feature, at least among nerve cells, and that their presence does not require, and is not brought about by, presynaptic cholinergic endings. If this is true for cholinceptive membranes, there is no reason to doubt its truth for sensitivity or responsiveness to other transmitter agents. It may be pointed out that many embryonic organs respond to the future transmitter substance (ACh), and to epinephrine, before they are innervated, as has been shown for cardiac tissue of rat and chick (197 to 199). For a further discussion of the problem see the section on denervation hypersensitivity (p. 520).

#### BIOPHYSICAL EVIDENCE

In many cases, biophysical measurements and data are best explained by the assumption of chemical synaptic transmission. Prominent among these are the studies of neuromuscular transmission in crustacea. Chemical transmission here was already indicated by the results of Marmont & Wiersma (200). The subsequent studies of Wiersma & van Harreveld (201, 202) led to the suggestion that the transmission of excitation from motoneuron to striated muscle fiber involves two processes, the first giving rise to junction potentials, the second giving rise to the process of contraction. Both were shown to be somewhat independent of each other inasmuch as the time courses of facilitation and fatigue of each process are different and obviously not directly causally related. Additional support for the assumption of a double process of transmission was given by the discovery of the "paradox" where stimulation of the fast motor fiber to the closer muscle of crayfish and that of *Blepharipoda* and *Randallia* gives rise to large junctional potentials which are not accompanied by contraction, while stimulation of the slow axon causes contraction without detectable junctional potentials; the latter show a slow rate of facilitation and eventually become

measurable (202, 203). The paradox has recently been confirmed by Hoyle and Wiersma (204) with the use of intracellular electrodes. Since both slow and fast motor fibers are supposed to innervate the same muscle fibers, it is fairly obvious that detectable junction potentials are not a prerequisite for subsequent contraction and that size of junction potentials is not related to the extent of resulting contraction. One is forced to consider the possibility that nerve fibers can have a direct action on the contractile mechanism which is independent of the action on the membrane. An important discussion of the problem can be found in (204). Further support of this is given by the fact that the action of the peripheral inhibitory fibers is a twofold one: it affects the excitatory junction potentials and it prevents contraction [for a comparative study see Wiersma & Ellis (205)]. These two types of action are again independent, as the action on junction potentials takes place only if impulses over the inhibitory fiber arrive shortly before the motor impulses, while mechanical inhibition takes place whether or not the excitatory junction potentials are altered [for a review of the literature until 1949 see Katz (206); the later publications and findings are discussed by Hoyle & Wiersma (207)]. Florey & Hoyle (208) have recently shown that in crab muscle (*Cancer magister*, closer muscle), inhibition of contraction takes place in the absence of significant changes in excitatory junction potentials, membrane potential, and membrane permeability [in the same muscle in the lobster *Homarus*, membrane permeability is definitely altered during stimulation of the inhibitory fibers (208, 209)].

The findings concerning neuromuscular transmission in decapod crustacea present a serious challenge to traditional concepts of synaptic transmission in general and the concepts of chemical transmission in particular. If it is true that nerve action on contractile processes is largely independent of the responses of the muscle fiber membrane, the conclusion seems inevitable that transmitters act not only on the postsynaptic membrane but actually below it within the postsynaptic cell.

There is no question the chemical type of transmitters can penetrate cell membranes (ACh may be an exception). This has been shown for catecholamines and epinephrine by Raab & Gigg (210), Axelrod *et al.* (211), and Muscholl (212). Elliott & van Gelder (213) have shown that gamma-aminobutyric acid is rapidly taken up by the cells of brain tissue slices.

The relaxing action of applied epinephrine on the mammalian intestine may well be caused by its intracellular action on metabolism [see Ramos (214); Mohme-Lundholm (215)]. How strongly and intimately metabolic processes going on just below the cell membrane affect membrane potential and impulse generation in nerve cells has in recent years been beautifully demonstrated by A. and N. Chalazonitis [ganglion cells of *Aplysia* (216 to 219)].

An intracellular transmitter action may well be involved in the case of the action of 5-HT on the anterior byssus retractor muscle of *Mytilus edulis* [Twarog (220)]. Application of small amounts of ACh or brief catho-

dal electrical stimulation causes a sustained contraction which cannot be reduced by washing and which may be maintained for hours (220). 5-Hydroxytryptamine ( $10^{-9}$  to  $10^{-7}$ M) causes immediate relaxation (220). In the presence of 5-HT the muscle contracts in response to ACh and DC current; in fact, the immediate response may be enhanced. The induced contractions are, however, not maintained, and relaxation occurs as soon as the stimulating agent is removed. The action of 5-HT is not accompanied by a change in membrane potential (220). Experiments (unpublished) carried out in my laboratory indicate that the action of 5-HT persists even after washing, indicating that the compound may well have penetrated the muscle cells. As Twarog suggests [see also Jewell (221)], it is most likely that the relaxing effect of the compound arises from an intracellular action. Whatever evidence there is available for a transmitter function of 5-HT has been discussed in the section devoted to this compound.

Another important recent development must influence our thinking about transmitter actions; Frank & Fuortes (222) have already presented suggestive evidence for presynaptic inhibition in the spinal cord. Eccles and co-workers have now conclusively proved that in certain cases inhibitory neurons make synaptic contact with presynaptic excitatory endings, thus reducing presynaptic activity (223). Eccles & Krnjevic (224, 225) and Curtis & Eccles (226) have furthermore shown that post-tetanic potentiation of transmission to motoneurons is a presynaptic event. These findings offer a new basis for the interpretation of facilitation phenomena which so far have often been ascribed to an accumulation of transmitter substances at the synapse. According to the new observations, facilitation can be explained by the release of larger and larger amounts of the transmitter with each successive impulse. It must be pointed out, however, that there are cases where facilitation must be assumed to be a postsynaptic phenomenon; heterofacilitation as described by Wiersma & Harreveld (202) is an example.

Most important in the study of the function and action of transmitter substances is the analysis of the changes in membrane permeability to various inorganic ions during transmitter action. During the past ten years tremendous progress has been made in this regard. Data obtained from vertebrates have been sufficiently reviewed elsewhere. Studies on crustacean muscle and stretch receptors have yielded much valuable information [Boistel & Fatt (227); Kuffler & Edwards (228); Edwards & Hagiwara (229)] and support the general conclusion that transmitters act by specifically altering the permeability characteristics of the subsynaptic membrane. The technical and theoretical advances of neurophysiology make any approach to the problem of chemical transmission obsolete that does not consider membrane permeability changes.

#### PHARMACOLOGICAL EVIDENCE

In recent years, several studies have been concerned with the action of chemical series of compounds on certain synaptic structures, nerve cells, and

effector organs. To these belong the extensive studies of Welsh & Taub (49 to 52) on the heart of *Venus mercenaria*; of Purpura and co-workers on the cerebral cortex of the cat (230); and of Edwards & Kuffler (181), Elliott & Florey (165), and McLennan (178) on the stretch receptor neurons of crayfish. The general pattern that emerges from these observations is that the action of a compound can be definitely related to its structure and that the specific activity of a structure diminishes with the extent of the alteration in molecular configuration. It is impressive that substitution of certain groups on a molecule with other radicals reverses the action of the compound or shifts its action from one anatomical substrate to another. For instance: substitution of a guanidino group for the amino group of gamma-aminobutyric acid transforms this compound from a "blockader of depolarizing axodendritic synapses" into a "blockader of hyperpolarizing axodendritic synapses". Substitution of the methyl groups of ACh by ethyl groups transforms the former into an ACh-blocking agent.

The findings are generally interpreted as indicative of highly specialized receptor molecules in postsynaptic cell membranes and one is, of course, tempted to conclude that this specialization indicates a normal role of the agent which reacts most actively and specifically with the hypothetical receptor. In fact, the conclusion has been drawn so often that it now forms the basis for many experimental approaches to the question of synaptic chemical transmission. A critical review of the available data indicates that the conclusion is not logically supported by evidence. Three examples may suffice; in some cases the most effective action is exerted by a compound which does not occur in the organism. The tryptamine derivative with the most powerful accelerating action on the *Venus* heart is N,N-dimethyl-5-HT (231). This compound does not occur in *Venus*; thus the specificity appears directed towards a compound which cannot be the natural transmitter (or regulating hormone). Epinephrine and norepinephrine are the most potent of a series of related compounds causing expansion of melanophore pigment of *Crangon* (113), yet they do not occur in crustacea (104). Stretch receptors of crayfish (66) and endings of a variety of sensory neurons in vertebrates [see Witzleb (196) for further references] are stimulated by ACh, and this compound is more potent than other cholinesters—but ACh is not involved in the normal sensory mechanism.

That chemical specificity exists cannot be doubted, but the knowledge of it does not necessarily lead us to the transmitter which normally affects the chemically specific structure.

The pharmacological approach to the detection and verification of supposed transmitter substances has been complicated by many recent findings which are of great importance for the evaluation of experimental results. Apparently, the common notion that agents which block synaptic transmission or which potentiate (by inhibiting antitransmitter enzymes such as cholinesterase, amino-oxidase) act always postsynaptically is no longer valid. Recent studies by Riker *et al.* (232) lead to the conclusion that curare exerts

part of its action directly on the presynaptic motor terminals and that the facilitating action of anticholinergic drugs also takes place at this presynaptic site. Similarly, Balzer & Holtz (233) have demonstrated a presynaptic action of amino-oxidase inhibitors in the sympathetic ganglion of cat and guinea pig. The significance of presynaptic terminals as intermediates between the all-or-none structures of pre- and postsynaptic cells is illustrated by the observations of Frank & Fuortes (222) and of Eccles and co-workers (223) on presynaptic inhibition in the mammalian spinal cord, and by the findings of Eccles *et al.* (224, 225) that central posttetanic facilitation of certain spinal reflexes is caused by changes in the presynaptic terminals. Burn & Rand (234) interpret the sympathicomimetic actions of ACh and nicotine as ascribable to epinephrine or norepinephrine liberated from presynaptic stores. Direct actions of eserine and prostigmine which cannot be explained by their anticholinesterase activity have been described by several authors (237 to 239). How complicated the synaptic function of cholinesterase may be is indicated by experiments of Fehér (240): ACh which is perfused through the superior cervical ganglion of the cat is normally hydrolyzed to the extent of 7 to 33 per cent. During and after preganglionic stimulation, as much as 12 to 73 per cent is inactivated. The hydrolysis in the quiescent ganglion is inhibited by  $10^{-5}$ M di-isopropyl fluorophosphate (DFP), but during preganglionic stimulation reactivation takes place. This can be inhibited by  $5 \times 10^{-5}$ M DFP. In the former case it is assumed that only the pseudocholinesterase is inhibited while the higher concentration of di-isopropyl fluorophosphate supposedly inactivates the true cholinesterase. Fehér suggests that there is a barrier between the location of pseudo and true cholinesterase and that this is removed by preganglionic excitation.

In studying the actions of anticholinesterases and cholinergic drugs, one must keep in mind that these agents can interfere with active transport of ions, particularly of sodium, through biological membranes. A discussion of this and an intriguing hypothesis can be found in a recent paper by Koblick (241).

*Denervation hypersensitivity and transmitter specificity.*—Since the publication of the well-known book by Cannon & Rosenblueth (242) in 1949 on "the supersensitivity of denervated structures", there have been several new developments, some of which are immediately relevant to a discussion of chemical transmission. Axelson & Thesleff (243) have shown that in mammalian striated muscle the ACh-sensitive membrane which normally is restricted to the vicinity of the endplates [see also Miledi (244)] spreads over the whole fiber surface after denervation. This has been confirmed by Miledi (245) using frog muscles. It appears now that innervation exerts a restricting influence on the "chemically excitable" membrane and that denervation hypersensitivity does not arise from hypersensitivity of normally sensitive membrane but from a spreading of the sensitive membrane. Luco & Sanchez (246, 247) have described contractile responses of denervated facial muscles of cats to epinephrine and norepinephrine. The sensitivity of these muscles

to these amines is as high as that of the denervated nictitating membrane. At the same time the muscles become "hypersensitive" to ACh (248). Curare blocks the actions of (nor-) epinephrine and of ACh and, considering the molecular weight, the blocking activity is of the same order for both types of compounds. This indicates that epinephrine-like substances might act on the same receptor molecules as does ACh. A hypothesis of "bivalent" receptor substances has been suggested by Fotino & Stoculesco (249) who find that the identical actions of ACh and epinephrine on the mammalian spleen are both blocked by atropine, yohimbin, and ergotoxin.

Of greatest interest are the experiments of Vera, Vial & Luco (250) in which the adrenergic supply to the cat's nictitating membrane was allowed to degenerate and the organ was reinnervated by cholinergic fibers from the hypoglossal nerve. Denervation resulted in hypersensitivity to epinephrine. This disappeared after cholinergic reinnervation. The contractile response to stimulation of the twelfth nerve could be blocked by atropine but not by dibenamine. In contrast to the nerve endings of hypoglossal nerve fibers on skeletal muscle, the invading fibers lost their myelin and built up a terminal plexus comparable to that of the normal sympathetic end-plexus. It should be mentioned that the nictitating membrane normally is sensitive to ACh, although it is not normally innervated by cholinergic fibers (235).

Luco & Eyzaguirre (251) showed that the onset of fibrillation and the hypersensitivity to ACh resulting from denervation of the tenuissimus muscle of the cat occurs the sooner, the more distally the nerve is severed. Similar results were reported by Davidovich & Luco (252) for sympathetic ganglia after preganglionic nerve section at different distances from the ganglion. They showed that conduction failure occurs at the same time regardless of the position of the cut while the synaptic effects clearly depend on the length of degenerating nerve fibers. Whether or not it is the transmitter that is responsible for the trophic actions of nerve fibers is, unfortunately, not yet clear.

It is interesting that sensory nerve endings are generally sensitive to substances which have transmitter functions in synapses. In many instances the sensory structures are noninnervated and it may well be that their membranes behave like those of denervated cells on which no restricting influence is exerted by presynaptic structures.

#### CONCLUSIONS

The scarcity of available data which can be used to support the many concepts involved in the hypotheses of chemical synaptic transmission is possibly attributable to a general belief that chemical transmission, particularly by such well-known agents as ACh or norepinephrine, is already well established. Particularly in view of the complexities of the actual experimental results, much more probing investigations are needed to clarify the general picture. This is particularly true with regard to invertebrate animals in connection with which, excepting a few preparations obtained from



decapod crustacea, we know so little about chemical transmission. At the present time generalizations are not possible; we cannot even predict the responses of homologous organs of members of other species or different taxonomic groups to one and the same (presumed) transmitter agent from the results obtained in one species, and what we have learned from the frog does by no means necessarily apply to fish, and what is true for certain teleosts is not true for elasmobranchs. It is also not permissible to speak of specific phenomena of synaptic transmission as occurring "in crustacea," as is done so often, since even related species within one of the many diverse groups may show striking differences. What is needed is precise information obtained from defined species. This cannot be obtained without detailed biochemical and biophysical analyses. Application of drugs is a valuable method, but the results must be interpreted with the awareness of the possibility of presynaptic or intracellular postsynaptic action, taking into consideration multiple sensitivity of receptor molecules and multiple actions of the supposed transmitter agent.

The original concept of neurohumoral transmission has matured into the concept of chemical synaptic transmission. Meanwhile the picture of the synapse as consisting of "all-or-none" presynaptic elements and reactive postsynaptic structures has changed. We now recognize modifying influences on the store of transmitter substances in the presynaptic endings as an important part of synaptic function, realizing that the "all-or-none" character of the axonal impulse can be modified within the presynaptic terminals. In the near future we may have to recognize the importance of intracellular postsynaptic transmitter action.

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## MAJOR PROBLEMS IN MUSCLE PHYSIOLOGY<sup>1,2</sup>

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with

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In this chapter, a deliberate departure has been made from the trend—illusory anyway—of providing a complete annotated bibliography. Such a summary listing is, at most, useful for the specialist, who can accomplish the same search with the aid of the regular abstracting journals. References pertaining to muscle are now collected from the *Excerpta Medica* and are published as "Muscular Dystrophy Abstracts" by the Muscular Dystrophy Associations of America, Inc. There have also been several compilations in monographs and handbooks, such as those written or edited by Bourne (411), Hoyle (412), Ernst (283), and Weber (149), with the aid of which even very recent literature is available. Since, on the other hand, a number of broad and profound problems have emerged from the large and scattered literature, we have found it advisable to concentrate on a discussion of these, quoting the literature to the extent required, in a manner understandable to a nonspecialist.

Additional omissions were needed because of the decision to include only that part of the extensive biochemical literature which relates directly to physiological and structural problems. Because of the chapter on cardiac muscle in this volume, we have discussed properties of the heart only when directly relevant to our selected problems, and the same restriction has been applied to smooth muscle.

### METABOLISM AND ACTIVITY OF MUSCLE

Most studies on the metabolism of muscular tissues, or on enzyme systems derived therefrom, are primarily of biochemical interest. Our selection has been guided by the functional importance of each work, but some arbitrariness may have entered in our omissions.

Although the elucidation of the main pathways of carbohydrate and other energy-yielding metabolism may be regarded as one of the established classi-

<sup>1</sup> The survey of literature pertaining to this review was concluded in April 1960, but in a few instances more recent papers were included.

<sup>2</sup> Among the abbreviations used in this chapter are: ADP (adenosine diphosphate); ATP (adenosine triphosphate); and NTP (nucleoside triphosphate).

<sup>3</sup> In the section on Excitation and Its Link to Contraction.

<sup>4</sup> In parts of the sections on The Dynamics of Muscle and on Excitation and Its Link to Contraction. Dr. Abbott's address: Department of Zoology, University of California, Los Angeles.

cal accomplishments [see important reviews by Bücher & Klingenberg (1); Krebs *et al.* (2)], so that further work would be largely a matter of clarifying the underlying chemical mechanism, the subject has not yet been exhausted. On the contrary, there remain major problems: the regulation of the intensity of metabolism in connection with functional activity; and the mechanism of the reversal of catabolism in order to effect a resynthesis of reserve food consumed in previous periods of activity. Which form of metabolism occurs *in vivo*? In the classical period of muscle biochemistry, attention was almost exclusively focussed upon the enzymes of glycolysis. Physiologically, anaerobic activity is correlated with glycolysis, and an isolated stimulated muscle has a respiratory quotient of unity; but these facts do not mean that normal activity in the body is always or entirely covered by oxidative carbohydrate utilization. The metabolism of human skeletal [Andres *et al.* (3)] and cardiac [Bing (4)] muscle *in vivo* is only partially accounted for by carbohydrate oxidation, the remainder being presumably or demonstrably attributable to lipid metabolism. Further experiments *in vivo*, or on the utilization of various metabolites by isolated muscle [e.g. (5 to 10)], are of interest.

*Phosphate compounds.*—A presentation of their chemistry and enzymology can be left to biochemical reviews, as there are up-to-date summaries on the biochemistry of nucleotides (11 to 14) and phosphagens (15, 16).

We may take this occasion to discuss the meaning of the term "phosphagen", which in keeping with current and still broadening biochemical knowledge we could define as "a substance that is not a transitory intermediate on a major metabolic pathway, which can transfer a phosphate onto a nucleoside diphosphate by an enzyme present in the tissue of its occurrence." Ennor & Morrison (15) restrict their definition to N-phosphorylated guanidine derivatives, and as long as no other examples are known, the difference is academic. However, if other substances were to be discovered, the broader definition might be advantageous. In the latter context, the earlier Russian literature on phosphorylcarnosine was reviewed by Stekol (17). Parker & Langley (18) have not confirmed earlier claims on the effect of carnosine on metabolism of minced muscle. Seraydarian & Mommaerts (19) separated three components, the imidazole-, the  $\beta$ -amino-, and the di-substituted products. No final evidence as to their biological occurrence was obtained, while Cain *et al.* (20) have given elegant evidence against their presence in turtle muscle. These negative findings, however, do not in our opinion close the matter, especially not with regard to phosphorylserine, on which no work is published, and which we regard as more likely to be of biological importance.

*Regulation of metabolic rate.*—A special example of variation in metabolic intensity, attributable to aerobic versus anaerobic conditions, is the Pasteur effect and its counterpart, the Crabtree effect (the depression of respiration by glycolysis). Inasmuch as respiration is localized in mitochondria and glycolysis in sarcoplasmic enzymes, it would seem fruitful to study these effects in cell-free systems [first realized by Meyerhof & Fiala (21), for dis-

rupted yeast cells], and more specifically in reconstituted systems of glycolytic enzymes and mitochondria. Such investigations have been reported for liver mitochondria and soluble enzymes from brain or tumors (22 to 24), for liver mitochondria, fractionated glycolytic enzymes, and ascites tumor cells (25 to 27), and for liver mitochondria and muscle extract (28). Thus it has been possible to show that with an excess of nucleotide and with phosphate as a limiting factor, the oxidative and glycolytic systems compete for phosphate, and, with limiting ADP and excess phosphate, for ADP. This provides an experimental illustration of the validity of the concept that the availability of esterifiable phosphate, or of phosphate acceptor, respectively, can limit the metabolic rate [also demonstrated in the penetrating investigation of Chance & Williams (29) on the respiratory control of mitochondria and cellular systems] and so contribute to the Pasteur effect. Results do not, so far, permit a quantitative account of the effect in living tissue, nor do they exclude the participation of other mechanisms, as indicated by the work of Sissakian & Pinus (28). Furthermore, Kaye & Mommaerts (30) suggested that the Pasteur ratio may depend on the ionic environment of the cell membrane, the biochemical mechanism of which has not yet been formulated.

Before discussing which metabolic reaction is rate-limiting, it may be useful to point out just what the assumption of a rate-limiting step means. In a multienzyme system in the stationary state, every step proceeds at the same speed, implying that each intermediary substrate must be present in a concentration relatable to the Michaelis constant and maximal velocity of its enzyme [Nanninga & Mommaerts (101); cf. Hohorst *et al.* (31), for an important study on metabolite concentrations in liver]. If a given step even at maximal substrate saturation is still slower than the rate of preceding reactions, no over-all steady state will be established, but its substrate will continue to accumulate indefinitely, or until the situation changes, or until an equilibrium mixture arises. Since the former contingency, especially, does not seem to occur, one is led to expect that a stationary metabolic situation such as resting glycolysis is limited at the first step, i.e., at the phosphorylase level. There are many indications of this, and Cori (32) has enunciated his important concept that this is ascribable to the predominant occurrence in resting muscle of phosphorylase in the inactive *b* instead of the active *a* form. In gross outline, the low *a* content in resting muscle has been confirmed [Krebs & Fischer (33)]. However, our current work on the phosphorylase *a* content in completely resting frog muscle interrupted with the rapid freezing method shows that this content is still too high to explain the low glycolytic rates [Guillory & Mommaerts (34)]. There are strong indications that the true inorganic phosphate content is much lower than was hitherto believed, and this may then contribute an additional limiting mechanism [Seraydarian *et al.* (35)], the more so if a sizable fraction of such low remaining phosphate might be bound by protein. In activity, both the phosphorylase *a* and the true phosphate may increase and permit a higher intensity of metabolism, and in that condition, another reaction may become limiting. Neifakh &

Melnikova (36) studied rate-limiting factors in glycolysis in muscle extracts. The chemical mechanism of the activation of phosphorylase *b* to *a* in a reaction involving ATP and phosphorylase kinase is being investigated in several laboratories, but this problem will be left to biochemical reviewers (Stetten & Stetten 37), except for the finding by Krebs *et al.* (38) that phosphorylase kinase itself may be inactive in resting muscle and may specifically require calcium for its activation. Because of the presumable role of calcium in the excitation of muscle (p. 565), this finding opens interesting questions on the relation between stimulation and the activation of metabolism.

Recent investigations on insect muscles are of particular interest. Some of them are capable of exceedingly high rates of metabolism in active flight. For predominantly biochemical investigations, reference is made to the summaries by Sacktor (39) and by Bücher *et al.* (40). It is possible that here respiratory control may be exerted not only by the availability of phosphate or phosphate acceptor, but also by the availability of an essential divalent cation. The problem of the  $\alpha$ -glycerophosphate cycle is mentioned under a later heading.

*Morphology and biochemical organization.*—In a carefully conducted investigation, Hill (41) attempted to determine the localization of ATP in the sarcomere by autoradiography of tritium-labelled adenine incorporated by previous administration. Despite all possible efforts, no clear resolution was achieved, but there may be a high concentration in the boundary between A- and I-bands, a region which may be of special interest (218, 219).

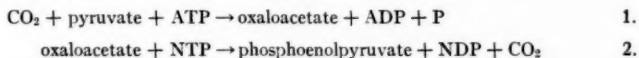
The biochemical and structural organization of three contrasting types of insect muscles has been treated in an investigation from Vogell and Bücher's laboratories [Vogell *et al.* (42)] which we consider a model among correlative investigations. It is true that differences like those reported can be discerned in the distinction between red and white muscles among vertebrates as well, but the comparisons made here are unusually instructive. The flight muscles are rich in mitochondria and oxidative enzymes and have a meshwork of intracellular tracheoles. The extensor tibiae involved in occasional jumps consist mainly of fibrils, but are also excessively rich in lactic dehydrogenase. In the tonic flexor tibiae, the relatively few mitochondria are typically placed near the Z-membranes.

*Other pathways of carbohydrate metabolism.*—The discussion of alternate pathways will be left to reviews of biochemistry, but mention must be made of recent interest in the metabolic role of  $\alpha$ -glycerophosphate. Glycerophosphate may be significant in relation to fat oxidation, although Bücher *et al.* (40) remind us that, only a small part of a fat molecule being represented by its glycerol moiety, this would quantitatively be of minor significance. Most work deals, therefore, with the place of glycerophosphate in carbohydrate metabolism, as found in insect muscle [Sacktor (39); Bücher *et al.* (40)]. Here, lactate formation is of minimal significance; instead there is a dismutation of triosephosphate into glycerophosphate and pyruvate. There are species differences in the extent to which these accumulate or are oxidized, but it is

clear that some form of the glycerophosphate cycle instead of the corresponding lactate cycle is the prominent pathway here. While this specifically applies to insect muscle, it is important that these results will be valid in a different way for vertebrate muscle as well. Bücher *et al.* (40) established that after tetanic stimulation of rat diaphragm, there is formation of equimolar amounts of pyruvate and phosphoglycerol, besides larger amounts of lactate. Also, we must remember how in the classical papers elucidating the pathways of glycolysis and fermentation, a period of "Angärung" was often distinguished in which a dismutation took place before the steady formation of lactate or alcohol was established; and how Lundsgaard (43) found that in anaerobic restitution the gain of phosphocreatine exceeded the now recognized theoretical value in proportion to lactate formation, because, we believe, part of the oxidation of phosphotriose was balanced by reduction of the same substance instead of pyruvate.

*Reversal of catabolism; glycogen synthesis.*—While it has long been known that during recovery after exercise a resynthesis of glycogen takes place, whether in the muscle itself or in the liver as is accepted for the intact mammalian organism, there is now, for the first time, considerable progress in the explanation of the mechanism of this reversal. Most of the reactions of glycolysis are freely reversible, and may be assumed to be driven in the opposite direction by a corresponding adjustment of the relevant substrate concentrations. There are, however, some steps in which this is probably or evidently impossible. These have been listed in an important lecture by Krebs (44) as reversal of the phosphofructokinase and pyruvate kinase reactions, to which we must also add the phosphorylase step. With the former, it is commonly assumed that fructose-diphosphatase takes care of the formation of fructose-6-phosphate, although to our knowledge its occurrence and activity in muscle have not yet been studied.

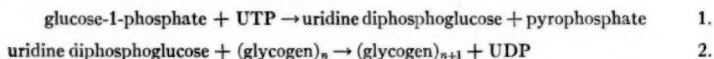
The formation of phosphoenolpyruvate from lactate or pyruvate was first tentatively ascribed to the Utter-Kurahashi cycle which, however, did not provide an energetic advantage except on the basis of appropriate concentration differences of the DPN and TPN reactants, i.e., a predominance of TPNH and of DPN. A new contribution by Utter & Keech (45) announces the discovery of a new pathway for the formation of oxaloacetate, so at the expense of two molecules of nucleoside triphosphate, rather than one, the formation of phosphopyruvate is now easily accounted for:



This is undoubtedly an important mechanism, although its occurrence under physiological circumstances is still to be investigated, and a full elucidation of the new reaction 1, with respect to the role of coenzyme A, will be needed for a final appreciation. Krimsky (46) showed that under certain circumstances a spontaneous reversal of the pyruvate kinase reaction is demonstrable; but with a separate mechanism provided by the Utter & Keech cycle,

it is more likely that the latter will turn out to be the preponderant pathway. It is of interest however, that according to Krinsky (47) the 3-phosphoglycerate kinase reaction is no more reversible than phosphopyruvate breakdown; his demonstration that this, too, can be made to proceed in the opposite direction by the proper choice of reactant concentrations may therefore be an essential contribution, since there are no indications of a bypass at this point.

Although the phosphorylation of glycogen is a textbook example of reversibility, it has recently become clear that this does not automatically account for glycogen synthesis. On the one hand, it is unlikely that glucose-1-phosphate ever occurs at the required concentration; on the other hand, one gains the impression that whatever one does to activate phosphorylase, the result always seems to be enhanced glycolysis [Sutherland (48); Stetten & Stetten (37)]. As was first demonstrated by Leloir & Cardini (49) for liver, glycogen synthesis at the expense of a nucleoside triphosphate is accomplished by the following reaction sequence:



Indications of the occurrence of this pathway in muscle were obtained by Villar-Palasi & Larner (50), by Robbins *et al.* (51), and by Hauk *et al.* (52, 53). Here, too, the participation of the pathway under physiological conditions remains to be elucidated, but an interesting argument for its importance has resulted from the study of a muscle disease caused by the absence of phosphorylase, in which the enzyme for reaction 2 is present and a sizable accumulation of glycogen occurs [Mommaerts *et al.* (54); Schmid *et al.* (55); Larner & Villar-Palasi (56)]; since glycogen cannot be mobilized in this disease, its obvious functional symptom is an extreme fatigability.

*Nitrogen metabolism.*—Developments in other areas of metabolism suggest the investigation of the reamination of inosine monophosphate, and an important step in that direction was made by Yefimochkina & Braunstein (57) and by Newton & Perry (58) who demonstrated a muscle enzyme catalyzing this reaction with aspartic acid, with adenylosuccinate presumably as the primary product (58). A study on glutamine metabolism [Ferdman & Silakova (59)] is also referred to.

Before and during the review period there has been increasing interest in the study of the turnover of tissue proteins from two points of view: the incorporation of labelled amino acids into whole muscle proteins as affected by insulin and other factors, and the turnover of individual pure protein components. We must leave a discussion of these topics to biochemical reviews, but wish to single out a few of the contributions from Schapira's laboratory, which are of more direct physiological interest. Kruh *et al.* (60) have studied the metabolism of myosin and of water-soluble proteins in normal mice and those afflicted with a hereditary muscular "dystrophy", with results interpretable as a shorter life span of parts of the muscle cell, or

as an acceleration of the turnover of its proteins. Dreyfus *et al.* (61) studied myosin after administering a single dose of labelled glycine and found that this, unlike aldolase which shows the behavior indicative of protein turnover [Schapira *et al.* (62)], displays a plateau of constant radioactivity. This was not found under the conditions of a high-protein diet, so that the problem may require further clarification. If accepted, however, the evidence leads to the important concept that myosin, like hemoglobin, does not engage in a steady turnover, but has a limited lifetime of about 30 days, indicative perhaps of a limited lifetime of the fibrils.

*Chemical reactions in contraction.*—The problem of the chemical nature of the "ultimate" energy-yielding reaction has been with us for generations, and most readers will be familiar with the historical development from inogen through lactate via creatinephosphate to the ATP theory [cf. Mommaerts (63 to 65)]. The foundation of the ATP theory is most impressive; indeed it differs from all previous theories because it does have a foundation. Yet, scientific rigor requires a direct demonstration of a breakdown of ATP as a reaction immediately connected with contraction or relaxation, and, this not having been made, the theory is in a most peculiar position. The immediately relevant investigations date back a few years [Fleckenstein *et al.* (66, 67); Mommaerts (68, 69)], but it may be reiterated that, in technically different ways, both attempts to show the expected chemical changes in a twitch were unsuccessful. Still, especially in the experiments developed in our laboratory, the utmost care was taken to assure immediate freezing of the muscles at low temperature and extraction of the tissue after powdering without secondary errors, methods which are constantly being improved [Seraydarian *et al.* (35); Mommaerts (70)]. We must first discuss some more recent efforts along this line. A claim was made by Wajzer *et al.* (71), based upon a change in ultraviolet transmission, that ATP is dephosphorylated in a twitch. The following objections must be recorded: it is not certain that the changes are caused by absorption changes since we are dealing with a heterogeneous scattering system in a wavelength region where anomalous dispersion may well occur; the records show changes, but no indication of how much change one may be dealing with; and, if real, the reported changes are indicative of deamination and allow no conclusions as to dephosphorylation. In a later paper, Wajzer *et al.* (72) report formation of inosine monophosphate in a single contraction, but the interruption of activity by mincing requires about a second and can hardly be a reliable procedure; even in more prolonged contraction, inosinic acid is not believed to be formed.

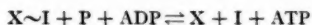
This problem deserves presentation from a somewhat broader viewpoint. First of all, a breakdown of ATP might well be immediately reversed by transphosphorylation from phosphorylcreatine, but we believe we have excluded this, too, at least for single twitches and very brief tetani. Incidentally, this transphosphorylation can take place, as was classically supposed, in iodoacetate-poisoned muscle. Ennor & Morrison (15), in their significant review on the chemistry of phosphagens, pointed out that creatine



phosphoryltransferase is about as susceptible to iodoacetate inhibition as is phosphoglyceraldehyde dehydrogenase, so that in the poisoned muscle the transferase reaction should be inhibited as well. But Carlson & Siger (73) and Padieu & Mommaerts (74) demonstrated that no such inhibition occurs in the whole muscle, and the latter authors ascribe this to a protective action of its substrates, mainly ATP and phosphorylcreatine, which is demonstrable *in vitro*. The question is then: could the use of ATP in a single twitch have been covered up by a phosphoryl-reservoir other than phosphorylcreatine? There is no indication of the occurrence of any other guanidinophosphagens, and a bulk occurrence of phosphoenolpyruvate was excluded by Mommaerts (69); our evidence on 1,3-diphosphoglycerate is inconclusive, but it is unlikely to accumulate in resting muscle because of a mutase [Joyce & Grisolia (75)], which would form 2,3-diphosphoglycerate. The possibility of a phosphorylated carnosine has been alluded to in a previous section. So far, then, there have been no consistent positive indications of any additional phosphate reservoir.

Certain results obtained in Chance's laboratory are highly relevant. Using their ingenious and carefully evaluated spectrophotometric recording techniques of the oxidoreduction state of respiratory chain components and their known responses to the ADP level, Chance, working alone (78) and with associates (76, 77), concludes that only about  $0.005 \mu\text{M}$  per gm. of ADP is formed in a twitch. This amount is real, and may well be crucial for the respiratory control of the cell; but it is only one or a few per cents of what would be energetically sufficient. These investigations, together with the chemical approaches mentioned above, leave no other conclusion than that the primary breakdown of ATP in contraction remains to be demonstrated. It is possible, however, that current work in Davies' (79) and our laboratories may profoundly alter this situation and throw a completely new light upon the matter.

Assuming, meanwhile, that no such development takes place, how would one then reconcile the evidence obtained from living muscle with the broad and impressive foundations of the ATP theory? Much can be learned from current developments in aerobic phosphorylation [Slater (80); Chance & Williams (29); Green (81); Lehninger (82)] in which one deals with unknown intermediates X and I, which generate ATP in a reaction presentable as:



which can be followed by



either spontaneously or stimulated by dinitrophenol, in which case the two reactions would jointly have an "ATPase" effect (it has been emphasized repeatedly [Mommaerts (83)] that ATP splitting in a cell need not be due to an ATPase as a direct hydrolytic enzyme). That myosin-ATPase can, under certain conditions, be activated by dinitrophenol (p. 540) could give rise to

the thought that the structural proteins themselves might contain groupings equivalent to X and I, through an interaction of which a limited contractile activity could occur uncompensated, although normally it would, sooner or later, be coupled with ATP breakdown. According to such a view, even a final and rigorous proof of a contraction without phosphate metabolism would not be completely at variance with the great discoveries that led to the ATP theory. It is not unlikely that one of the reactive groups would be a sulfhydryl, and it is of interest that Brahms (83a) has made a first effort to study changes in protein-SH in contraction.

We conclude with disappointment that studies on the turnover of phosphate in the phosphorylated metabolites have not contributed decisive information. The most satisfactory investigation is that by Fleckenstein & Janke (84), who reported that the incorporation of  $P^{32}$  into the terminal  $\gamma$ -P of ATP, while dependent on temperature, is not increased in activity. There is reason to believe that the  $\gamma$ -P is in full isotope equilibrium with labelled inorganic phosphate to the extent that the latter enters, so that no further incorporation is expected, unless by altering the temperature one also alters the entry; the observed turnover in the second P of ATP is indicative of enhanced participation [Hasselbach (85)]. Secondly, Fleckenstein & Janke (84) found a difference in specific activity between phosphorylcreatine and the  $\gamma$ -P of ATP, which difference is not reduced in activity as would be expected when ATP is cyclically resynthesized from phosphorylcreatine. This point is experimentally well established, but is open to various interpretations, such as a compartmentalization of ATP. The same ambiguity in interpretation afflicts the work of Dixon & Sacks (86), in which the variability of the results is so great as to preclude a clear statement. In the work of Sacks & Cleland (86), the point seems demonstrated more convincingly, but the situation in a stationary state in intermittently contracting respiring muscle is harder to evaluate. We believe, therefore, that these investigations have not provided explicit answers. More success may be forthcoming from studies with  $O^{18}$  incorporation which, administered as  $H_2O^{18}$ , rapidly labels the inorganic phosphate. In their significant initial study, Fleckenstein *et al.* (87) demonstrate an uptake of labelled phosphate into ATP and phosphorylcreatine during the recovery phase after contraction, in line with current concepts of restitution metabolism. During contraction itself, there was no such incorporation, but inasmuch as the creatine phosphoryltransferase reaction does not involve inorganic phosphate, this result still does not contradict the classical concepts.

In more prolonged activity, of course, and this is best investigated on the iodoacetate-poisoned anaerobic muscle, there is an analytically demonstrable utilization of high-energy phosphates, which appears as a splitting of phosphagen without initially reducing the ATP [Carlson & Siger (88)]. These workers found that the progression of phosphagen breakdown, if extrapolated back according to the most probable line, would indicate that the first two twitches took place without any phosphorylcreatine splitting; however,

the scatter of the results was such that a splitting from the first twitch on would also be compatible with the findings (and this would not be contradicting our single twitch results, in which it was not excluded that after a contraction cycle the expected changes might well occur). Carlson & Siger (88) also conclude that throughout the progression of exhaustion, ATP, phosphorylcreatine, and creatine stand in equilibrium with each other, but that the ADP concentration is unexpectedly high. This discrepancy may well be related to the bound ADP in the fibrils [Perry (122)].

While the nature of the primary reaction must await further work, we can, meanwhile, use measurement of the total change of phosphorylcreatine in a series of contractions of iodoacetate-poisoned muscles, to investigate the chemical basis of the regulation of energy release. This will be discussed in a later section under Energetics of Contraction.

#### THE CONTRACTILE STRUCTURE AND ITS COMPONENTS

In this section, much purely chemical material had to be omitted. Our choice of investigations to include may have been erratic, but was guided by an attempt to judge either the more crucial importance, or the possible functional or morphological implications of such work.

*Myosin.*—The molecular weight of myosin, for some years considered to be 850,000, has now been revised downward and may be established reliably in the 450,000 region. The first doubts about the quoted value, established by ultracentrifugation and diffusion, were expressed as a result of earlier findings in our group that the sedimentation behavior showed anomalies; this was criticized by Schachman (89), but then confirmed by Johnson & Rowe (90), and ascribed by them to aggregation. Meanwhile, investigators employing the light-scattering method had obtained lower and lower values; most recently Holtzer (91), and Holtzer *et al.* (92 to 94) reached about 490,000. Lowey & Holtzer (95) consider the molecule to be made up of one unit each of heavy and light meromyosin, of which they determined the molecular weights at 329,000 and 126,000, respectively, in good agreement with unpublished light-scattering studies by Gergely. What may turn out to be the final value for myosin was first obtained by Mommaerts & Aldrich (96) for pure myosin from an interferometric study of the approach to equilibrium sedimentation-diffusion in the ultracentrifuge using the Archibald theory, with mol.wt. = 420,000 as the outcome, obtained both from the meniscus and the bottom of the cell; and by Von Hippel *et al.* (97) for the lightest component in actomyosin systems, with some associated measurements on purified myosin. Both studies were somewhat exploratory in character, and further intensive effort may well reach greater accuracy, the uncertainty at present being perhaps 10 per cent; we tend to accept 450,000 as a working value, equal to the sum of the molecular weights of the subunits according to Holtzer, and slightly below his measured molecular weight. Unfortunately, the same experimental approach in the hands of Kielley & Harrington (98) gave a considerably higher result, 630,000; these workers

did direct measurements on the concentration dependence of the molecular weight, which they found more pronounced than was previously believed.

In the meantime, other approaches also indicated an entity of about mol.wt. = 420,000 to 450,000 as the reactive if not the particulate unit. Nanninga & Mommaerts (99), in an evaluation of work by Mommaerts & Hanson (100) on the turbidimetric response of actomyosin solution to stoichiometric amounts of ATP maintained by rephosphorylation with pyruvate kinase, found that 1 mole of ATP reacted with 507,000 gm. of actomyosin, presumably containing 420,000 to 450,000 gm. of myosin. More directly and accurately, Nanninga & Mommaerts (101, 102) then studied the binding of ATP to pure myosin, again in the presence of a rephosphorylating enzyme, with their luciferase method (see below), and found about 430,000 for the reacting equivalent weight of the protein. Finally, Gergely *et al.* (103, 104) found this same proportion for the binding of pyrophosphate, although Tonomura & Morita (105) reported a different proportion for this same reaction. Besides confirming the presumable magnitude of the molecular weight, these demonstrations also indicate that myosin does not react with an amount of ATP large enough to produce a diffuse negative charge, such as was invoked by Morales *et al.* (106) in an electrostatic contraction and relaxation mechanism, a difficulty meanwhile recognized by this author (107) as well.

There continues to be dispute about the nature of the response of actomyosin particles in solution towards ATP [e.g. (108 to 110)]. A majority of earlier investigations, beginning with the less direct demonstration by Mommaerts in 1945, had indicated that this response consists of a dissociation of the complex into its actin and myosin components, but Blum & Morales (111) concluded from a light-scattering analysis that, instead, an elongation of the particles took place, a proposal at variance with the evidence from sedimentation, viscosity, and other studies. Gergely (112) has shown that light-scattering work also supports the disaggregation theory, but whether there might not be a remnant of effects requiring another additional interpretation is still being discussed [Von Hippel *et al.* (113); Morales (107)]. Flow birefringence studies [see also Kalamkarova (114)] also indicate dissociation, except for some unexplained anomalies [Noda & Maruyama (115); Maruyama (116)]. It has always been the reviewer's opinion that the phenomena are caused by disaggregation, but there may indeed remain some points of detail that require an explanation, especially in impure systems.

*Myosin-ATPase.*—Most studies on the chemistry and enzymology of the structural proteins are too chemical to be mentioned in this review, but a few papers are of sufficient impact to warrant their inclusion. We wish to call attention to Kielley & Bradley's (117) observation that under certain conditions *p*-chloromercuric benzoate, in half the amount equivalent to all free sulfhydryl groups on myosin, causes a strong stimulation of ATPase instead of the usual inhibition; an explanation of this interesting phenomenon might involve the assumption of two SH-groups at each active center able to com-

bine with ATP, one giving an enzymatically active configuration, the other an inactive one, the latter combining preferentially with the mercurial. Considerable interest is attached to the activation by dinitrophenol, discovered by Greville & Needham (118) and Chappell & Perry (119); this was recently studied by Blum & Felauer (120) who explored its effect upon the hydrolysis of several nucleoside triphosphates and with respect to different aspects of the kinetics and arrived at the tentative conclusion that the agent acts in part by affecting the interaction of the purine or pyrimidine ring of the nucleotide with the active site. In a broader context, this problem deserves attention because of its possible relation to the effect of dinitrophenol in oxidative phosphorylation (p. 531).

Studies by Koshland *et al.* (121) on the temperature dependence of myosin-ATPase showed that this follows a single Arrhenius plot, but is changed into a broken curve under the influence of either actin or dinitrophenol. The biphasic line has a remarkable similarity to the plot of the well-known Shapley data on the walking rate of ants, and the authors do not hesitate to regard this as indicating a direct connection between a kinetic property of actomyosin and contraction velocity. The obvious objection that the walking rate may be determined by a nervous rhythm may not apply; we believe there may be a feedback connection which would keep the contraction velocity in the rate-limiting position.

Kinetic studies on myosin-ATPase as such have not, in general, been able to cope with the enormous diversity of responses shown by this enzyme to the electrolyte medium, to specific activation and inhibition effects by magnesium and calcium ions and other modifiers, and to the presence of actin in actomyosin or in more highly organized systems. A review by Perry (122) touches upon some of these aspects, from which we single out this author's contribution that magnesium may be less inhibitory to the ATPase activity of fibrils than of myosin (its action is still greatly dependent on the ionic strength), so that the possible activity of this ATPase *in vivo* remains difficult to estimate and may not be as low as has been thought [Mommaerts (123)]. The magnesium-activated ATPase is also very sensitive to excess-substrate inhibition [Perry (122)]. Clearly, there is need for a complete study of the effects of all relevant ions upon all kinetic constants in myosin as such and in more complex systems, a well-nigh impossible task. Among partial approaches to this problem is that by Nanninga (124) dealing with the calcium-activation of myosin-ATPase, which is satisfactorily accounted for by assuming that calcium myosinate (specifically, of course, with respect to a binding group near the active site) is the enzyme, free ATP the substrate, the Ca-ATP an inactive substance.

A new approach to the kinetics of myosin-ATPase was initiated by Nanninga & Mommaerts (101, 102) by a methodology in which free ATP concentration in a system was continuously monitored by recording the luminescence emitted by admixed luciferin and luciferase, and in which ATP was kept fully phosphorylated by creatine phosphoryl transferase. This served

to determine the stoichiometry of binding referred to above, the Michaelis constant from a binding curve, and the formation constant  $k_1$  of the enzyme-substrate complex. These studies confirmed the impression gradually formed (Mommaerts, 1948) that the binding of ATP to myosin has a considerable energetic effect, and further showed that  $K_M$  practically equals  $k_3/k_1$  as experimentally determined, and so is not an equilibrium constant under the conditions of the experiment.

A kinetic phenomenon of great interest was discovered by Weber & Hasselbach (125) and by Tonomura & Kitagawa (126): upon adding ATP to actomyosin, there is a fast initial rate of splitting, which after a few seconds settles to a slower steady-state velocity, a phenomenon that can be repeated upon renewed addition of more ATP. The two-phase course depends on the composition of the medium. Tonomura & Kitagawa (127) have now demonstrated that the effect is abolished by dialysis of the myosin, and is restored by  $Mg^{++}$ , but not by  $Ca^{++}$ . No explanation is as yet agreed upon; in absence of more specific information as to the mechanism of the phenomenon, it might fall in the category of effects that could be ascribed to a configurational change in part of the protein molecule [cf. Koshland (128)]. Weber & Hasselbach (125) attempted to connect this phenomenon with the gradual increase in economy of tension maintenance occurring in the course of a tetanus.

*Actin.*—Among its unsolved chemical properties is the nature of the participation of its SH-groups in the polymerization process. We had originally proposed that one such group per molecule engages in the formation of a bond analogous to the reactivity of coenzyme A, but all properties of the SH-groups were so anomalous that no rigorous demonstration of the disappearance of one such group ever succeeded. Bárány *et al.* (129) concluded that two such groups are consumed in the bond formation, but no critical tests for the validity of the analytical procedure were reported. Bárány *et al.* (130) contributed an improved preliminary step in the preparation of actin and offered evidence that in muscle this protein occurs in the polymerized state.

Strohmman (131) studied an interaction *in vitro* of actin and its associated nucleotide and creatine phosphoryl kinase. It is reported that the ADP previously found to be strongly bound to fibrous actin (Mommaerts, 1952) is not accessible to rephosphorylation and is, therefore, tightly bound indeed. Regarding ATP associated with globular actin as such or in the presence of H-meromyosin, it is concluded that this is also bound completely, but can react with external enzymes while in this condition. This latter conclusion is opposed, while the former is confirmed, by Martonosi, Gouvea & Gergely (132) in an investigation of the exchange of  $C^{14}$ -labelled nucleotides. G-actin exchanges promptly, but F-actin not measurably. Our experience would make us side with the Gergely group in this regard; we have found that the ATP in G-actin can rapidly be replaced by other nucleoside di- and triphosphates. Presumably, although G-actin binds such nucleotides, their free concentration is sufficient to permit their reaction with added enzymes; however, a direct exchange reaction of one nucleotide for another might ex-

plain this. In a second paper [Gergely *et al.* (133)], these authors studied the nucleotide exchange phenomena in partly polymerized actin and found evidence against the assumption of a critical concentration of G-actin above which polymerization occurs [Oosawa *et al.* (135)], and against the view that there is a stationary state of reversible transitions leading to a steady splitting of ATP [Asakura & Oosawa (136)]. Finally, the same investigators [Martonosi *et al.* (134)] studied the  $P^{32}$ -turnover of the nucleotide associated with actin *in vivo* and found no evidence of a repeated polymerization and depolymerization in contractile activity. Because of the complexity of the experiment, this conclusion may not be final, but it constitutes the only evidence available, and the burden of proof is now upon those who wish to defend a participation of the reversible polymerization of actin in the contractile event.

*Other muscle proteins. Paramyosin.*—We must leave to biochemical reviews the discussion of the discovery of several minor muscle proteins, and of other biochemical investigations in the protein field. We make exception, however, for work on paramyosin, in view of its physiological implications. Paramyosin is now also being called tropomyosin A, because of some features of its composition. The protein has been crystallized from the tonus muscle of bivalves and studied by several groups (137 to 143). It has a molecular weight of about 130,000 and a length of 1400 Å. Locker & Schmitt (144) and Hanson *et al.* (145) find a periodic banded structure in these crystals, similar to that in paramyosin filaments. Both the Bailey group and Johnson relate this protein to the maintenance of tension by the "catch" mechanism, as will be discussed under Dynamics of Muscle (pp. 536, 537).

*Contraction of fiber systems.*—The discovery of protein systems and of extracted muscle fiber preparations contracting with ATP [Szent-Györgyi (146)] and their detailed study by the Weber school (147 to 149) have been of great significance during the years preceding the review period. Weber's studies resulted in the recognition of the following distinction: a number of substances such as pyrophosphate, ethylenediamine tetraacetate, and certain sulfhydryl binding reagents, cause a fiber bundle to soften, and are called plasticizers; some substances, specifically ATP and other nucleotide triphosphates (NTP), also have this property and, in addition, cause contraction by a more specific interaction with the myosin component [e.g. Hasselbach (150)]. Weber considers this latter interaction to be a hydrolytic splitting of the NTP, so that the process of contraction depends upon a steady flux of energy besides a required plasticizing action. By contrast, it is implied by the views of Mommaerts (123), Morales *et al.* (106), and Weinbach & Bowen (151) that the binding of NTP to the myosin by itself can supply this energy. With respect to a related phenomenon, the dissociation of actomyosin in solution at high ionic strength by ATP, Nanninga & Mommaerts (102) concluded from their kinetic analysis that binding, not splitting, is the essential point; but for the contraction phenomena in organized systems, no such decision has been made.



Further studies on the contractility of such models appeared during the review period, some of which are summarized as follows. Adenosine tetra- and pentaphosphates are split more slowly than ATP by various contractile protein systems; they have a lower plasticizing action and cause no contraction [Hasselbach (152)]. Contrary to an earlier report [Feuer (153)], it was not possible to obtain contraction in actomyosin systems in which the ATPase was inhibited by iodoacetate [Geske & Weber (154)]. Hasselbach & Ledermaier (155) and Needham & Williams (156) studied the contractility of extracted uterus muscle fibers; these contain no demonstrable relaxation-factor (156). Moos *et al.* (157) found that an ATP analogue with a terminal P-CH<sub>2</sub>-P sequence was not split and caused no contraction in extracted fibers; since this result may be ascribable to absence of binding, this interesting approach also gives no answer to the question. Pentachlorophenol inhibits both contraction and ATPase [Weinbach & Bowen (151)]. Edman (158) has given attention to the diffusion of added ATP into the fiber-bundle as a heterogeneous system, concluding that even into the single fibers diffusion is limited. With strictly isometric recording, tension development is faster than otherwise, but is still a matter of seconds in the thinnest preparations; the "intrinsic" velocity remains to be evaluated. The same author [Edman (159 to 162)] has studied the effects of zinc and other metal ions. Fiber preparations from failing hearts have a lower contractility than those from normal hearts (163, 164). Kaminer (165) reports that fiber contractility is not inhibited in heavy water. Bozler (166) studied the light-scattering properties of extracted fiber preparations. Goodall (167) has shown oscillating behavior in the contracting system. Hayashi *et al.* (168), Zaalishvili & Mikadze (169), and Kafiani & Poglasov (170) confirm that myosin without actin does not form contractile systems, as was stated in an earlier report from the latter group.

A valuable experimental investigation is that of Mandelkern *et al.* (171) in which x-ray diffraction and other evidence are used to support the role of a melting of crystalline micellar regions in the contraction mechanism, as proposed by Flory (172).

*The relaxation factor.*—More attention has recently been devoted to the relaxation of contracted fiber systems, a problem which arose when Marsh (173, 174) described a factor preventing the synaeresis of minced muscle and Bendall (175, 176) showed that this can also be demonstrated as an actual relaxation of contracted glycerol-extracted fiber bundles. This field has now assumed considerable interest, although its physiological implications are unsettled. The view arose that in the presence of a factor there is, on the one hand, an inhibition of ATPase activity, on the other hand, a prevention or reversal of contraction. This factor, active in the presence of ATP and magnesium, is strongly inhibited by calcium. Especially if one keeps the presumed role of calcium in excitation-contraction coupling in mind, it is attractive to speculate that in resting muscle the factor keeps both ATPase and contractility in check, but stops doing so when calcium is liberated in

the act of stimulation—a roundabout revival of Bailey's (177) original concept of the activation of ATPase by calcium. We believe, however, that this picture is still quite remote from the established facts.

Originally, a correlation was found between relaxation activity and the presence of enzymes that could resynthesize ATP from ADP, viz., adenylate kinase (178), creatine phosphorylkinase (179 to 182), or pyruvate kinase (183). When a report appeared on relaxation caused by carnosine phosphate [Goodall (184)], this was taken as an indication of its phosphate transfer onto ADP (actually, this work needs re-examination in the light of the multiple composition of carnosine phosphate [Seraydarian & Mommaerts (19)] and its possible action as a chelating agent). This connection between rephosphorylation and relaxation has now turned out to be misleading.

The newer developments in this area started with the important work of Kumagai *et al.* (185) and Ebashi (186). In fiber bundles briefly extracted with glycerol, several factors are retained; but upon extraction for several weeks, these are gradually removed or inactivated, and a simpler behavior results. Then, it is found that adenylate kinase and other phosphophrases do not cause relaxation, but that the simultaneous presence is required, with ATP, of two fractions, A and B. The former, prepared by differential centrifugation is essential. It resembles the Kielley-Meyerhof particulate ATPase and is, like this, destroyed by *Clostridium* phospholipase. There are, however, ways of destroying the relaxing action without affecting ATPase, and other ATPases were inactive (except for a slight effect of apyrase, perhaps a result of pyrophosphate formation). Hence, there is a specific relaxation factor, separate from the phosphophrases presumably present in fraction B. These latter, in turn, are not directly essential as such; they serve only to maintain a sufficient ATP concentration throughout the fiber. Working with isolated fibrils, in which the relaxation effect is studied in terms of a prevention of contraction, and in which provision of ATP by diffusion is more homogeneously maintained (187 to 189), one finds that creatine phosphoryl transferase or other such enzymes are not required; only the relaxation factor as such is needed and only this inhibits ATPase. It is this factor, specifically, which is inhibited by calcium [Bendall (176, 189)], and it was found to be recoverable in a microsomal fraction [Portzehl (190)] consisting of granules of about 1000 Å [Hasselbach (191)]. However, Briggs *et al.* (192) found that only a part of the relaxation activity of a crude extract was present after isolation of the particulate matter, full activity being restored by adding the supernatant solution, and full inhibition of relaxation by calcium ions was obtainable when both the particulate and the dialyzable fractions were present. The relaxation effect is inhibited by carnosine. Corresponding results, with respect to the participation of a cofactor and the counteraction by carnosine, were obtained for the inhibition of myofibrillar ATPase [Gergely *et al.* (193)]. There is a certain variability here: some particulate preparations required dialyzable factor, others were active without it, and active granules could be converted by heating so as to require a cofactor. It

would be desirable to know explicitly whether the same variations would also be found in the contraction-relaxation test. The cofactor can be replaced by pyrophosphate [cf. Bendall (178) and Bozler & Prince (195)], but this is not the natural cofactor, which is stable towards pyrophosphatase, is labile under conditions where pyrophosphate is not, and is absorbed on charcoal [Kaldor *et al.* (194)]. Kaldor & Gergely (196) found that pyridoxal phosphate in millimolar concentrations inhibits both myofibrillar ATPase and contractility (it is not explicitly stated whether it can cause relaxation of a fiber), and that this effect is reversed by a fivefold excess of carnosine. The ATPase inhibition is maximal at 6 mM ATP, is zero at pH 6 and complete at pH 9, and is effectively reversed by carnosine up to pH 7.5, less at higher alkalinity. However, these results are not presented in terms of a closer kinetic analysis, and in unpublished experiments in our laboratory Seraydarian found that carnosine often went further than a mere reversal of the pyridoxal phosphate inhibition and appeared to stimulate myofibrillar ATPase by itself. These interesting effects deserve further study.

The latest significant additions to the picture have just been announced by Parker & Gergely (197) and by Briggs & Fuchs (198), who found that during incubation of the relaxation factor with ATP a substance is formed which by itself causes instant relaxation. This may have a significant impact upon the field, since it is clear that microsomes cannot rapidly penetrate into a fiber preparation [Weber (199)], but must exchange some material with it.

While the application of these results to the situation in living muscle is far from clear, the tendency to ascribe to the relaxation factor system the role of suppressing spontaneous enzymatic and mechanochemical phenomena will be discerned. Such a concept of negative control, as Gergely (200) expressed it, is contrary to most familiar arrangements in machines, but we consider it well in line with what can be expected in many instances of metabolic regulation in which enzymes must be prevented from acting at their full speed rather than the opposite. And the demonstrated, although unspecified, effects of carnosine in this connection assume considerable scope in view of the role considered for this substance and for carnitine in cellular excitation by Hayashi (201), against which, however, some experimental objections have been raised [Friebel (202)].

*Immunochemical studies.*—Immunological methods are being used increasingly for investigations on the identity and specificity of muscle proteins, for example, in connection with such problems as the early appearance of contractile proteins in embryological development. We shall discuss only one special area, namely, the localization of myosin and actin within the sarcomere, as indicated by the place where fluorescent antibodies are attached. This has been studied especially in the outstanding work by Marshall and his associates (203, 204). The original attempts were directed at establishing the localization of myosin, and this was found in the A-band, in agreement with the views reached gradually on the basis of other infor-

mation. Actin was found in the I-band and extending into the A-band, also in agreement with existing views, but an additional area of localization was found in the M-line; however, these studies are still preliminary and the antigen used, and hence the antibody, may not have been homogeneous. Tropomyosin may be in the Z-line. Greater difficulties arose when separate antibodies for H- and L-meromyosin were applied. These attached to the A-band, but to different parts, predominantly the center and the ends, respectively. This would, of course, be inconsistent with the concept of the occurrence of these meromyosins as parts of one and the same myosin molecule, which is only about one-tenth of the A-band length, but the possibility remains that, for example, the myosin molecules are arranged in such a way as to expose different specificity-determining configurations in different sections of the A-band. Besides, the antigenic behavior of several components is complex, and the experimental results may yet undergo some modifications. However, Laki (205) believes that myosin may not occur as such in muscle but may be an artifact formed from actin, tropomyosin, and a third component. Although we do not consider this opinion as proven, we certainly regard it as an exciting unorthodox viewpoint, emerging from the extensive and valuable work of this author's laboratory on the chemical composition of muscle proteins which cannot be reviewed here. It may be anticipated that further work on localization problems and on the immunological individuality of the proteins is entering into a fruitful stage.

*Structural basis of muscle contraction.*—Considerable progress has been made in the study of muscle structure by electronmicroscopy and other means. Several recent summaries are available (206 to 210). We shall deal only with certain individual investigations of special functional interest.

With regard to striated muscle, brief mention is made of some studies on light diffraction patterns (211, 212), ultraviolet dichroism (213), and to a study on the connection of the fibrils to the tendon (214, 215). Huxley & Niedergerke (216) described their observations on isolated muscle fibers with interference microscopy, showing that length changes, whether active contraction or passive stretch, occur primarily as changes of the I-band without changing the A-band length. This was interpreted in terms of a sliding filament model based, on the other hand, upon the electronmicroscopic and optical studies by Huxley & Hanson (207, 217). According to these authors, the A-band contains an array of parallel rods in a hexagonal pattern, about 150 Å thick and composed of myosin. In the I-band, and projecting into the A-band, are thinner filaments composed of actin; the H-zone of the A-band contains myosin rods only, while otherwise the heavy and light rods overlap and are connected with cross-bridges. Length changes are ascribed to sliding of these interdigitating rod systems, and where extreme shortening occurs there is a piling-up of material, thus forming contraction bands. These views are based upon electronmicroscopic work of the very highest standards and have come to dominate current thinking on the structural basis of contrac-

tion. Nevertheless, work on this level is dependent on the fixation of the structural components in their original configuration. When, therefore, alterations in the technique give rise to different pictures, it is not obvious which is closer to the truth. Current work by Sjöstrand & Andersson-Cedergren [see (218, 219)] does indeed indicate such a situation, so that a final judgement will need to be postponed.

With respect to the structure of smooth muscles, there is great heterogeneity. Hanson & Lowy (209) offer the following classification. (a) Helical smooth muscle. These have "myofibrils" which follow a helical course and show the pattern known as oblique striation. (b) Paramyosin smooth muscle. Their cells contain filaments of paramyosin, up to  $0.1 \mu$  diameter. (c) Classic smooth muscle. This is the heterogeneous category which belongs to neither of the two special classes. None of the smooth muscle types displays, of course, the special separation of two types of filaments found in striped muscle, but current work by Hanson & Lowy (220) shows evidence that sliding mechanisms may be at work in these muscles as well.

#### THE DYNAMICS OF MUSCLE

While numerous publications deal with one aspect or the other of the mechanics or energetics of muscle activity, we restrict our discussion to those papers related to major currents of research on central concepts. This part of muscle physiology is often referred to as "classical", but it displays a lively interest in highly fundamental questions, and here and there points of contact with the molecular basis of contractility begin to occur.

*The series-elastic component.*—During the development of the concepts of the Hill school [e.g., Wilkie (221)], there gradually arose the view that, besides the effect of a parallel elastic factor, especially at greater length, the mechanical behavior of muscle is comprised of the interaction between a contractile and a series-elastic component, as elaborated upon in the following sections. We shall deal here with evidence directly applicable to the series elasticity.

In an experimental approach applied by Wilkie (222) and Jewell & Wilkie (223), a tetanically stimulated muscle pulls against a load which is first held until the active state is fully established, and then released: one observes a sudden upstroke followed by a further shortening. The extent of the first, and the velocity and extent of the second shortening, depend on the weight of the load, the second phase being determined by the force-velocity relation (see below). The initial upstroke is ascribed to the shortening of the series-elastic component stretched during the isometric phase of the experiment, and now permitted to shorten to its new loaded condition. Both this demonstration, as such, and the possibility of deriving from it a length-tension diagram of the series elasticity strikingly illustrate the reality of this component as a nearly undamped elastic element. The careful work of Jewell & Wilkie (223) has largely eliminated the spurious contributions by

the compliance of the parts of the attachments and apparatus, and shows that in the frog sartorius the elastic elements are stretched by about 2 per cent of the muscle length at full isometric tension.

The same mode of experimentation has been applied by Abbott & Mommaerts (224) to the cat papillary muscle. This myocardial preparation offers an experimental approach not easily available with skeletal muscle: making use of the staircase effect and other inotropic phenomena, one can greatly vary the contractile strength under constant environmental conditions. Under all such variations, the mechanical properties of the series elastic component remained unaltered, an important argument in support of its existence as a separate entity without independent physiological activity. Also, the series-elastic component is not changed in iodoacetate rigor [Aubert (259, p. 269); Maréchal, unpublished].

These two lines of approach should definitely establish the series elasticity as an individual mechanical element, but no prejudice is expressed as to its structural allocation; in fact, it may not be morphologically distinct but may represent a separate kinetic feature of physiologically active structures.

*The force-velocity relation.*—This was also reinvestigated by Jewell & Wilkie (223) and was identical whether obtained by release after tension development, as described, or directly from the initial isotonic shortening velocity, implying that full activity was reached early in the isotonic contractions. Among miscellaneous investigations on the force-velocity relation, we mention that of Schottelius & Schottelius (225) who find a diminished contraction velocity in vitamin E deficiency, and (226) under the influence of tetanus toxin. Contraction is slower in heavy water, with the same maximal tension [Goodall (227)]. Rosenblueth *et al.* (228 to 230) have studied several aspects of the dynamics of cat muscle *in vivo*. Mauriello & Sandow (231) find that the rigor response in iodoacetate poisoning can be described by a force-velocity equation like Hill's with different constants. Abbott & Mommaerts (224) encountered the difficulty in cardiac muscle that no complete force-velocity can be determined because the take-off time needed for lifting the heavier loads may exceed the active state duration, since no tetanus is possible. Measurements on preloaded contractions are not subject to this uncertainty, but introduce an additional variable, initial length. It is demonstrated, however, that the maximal shortening velocity at zero load can be validly measured under both conditions.

Aubert (259) has proposed another equation for the force-velocity relation, viz.

$$P = P_0' e^{-V/B} \pm F,$$

in which  $P$  = load,  $P_0'$  = maximal isometric tension,  $V$  = velocity of movement,  $B$  = a constant, and  $F$  = a constant internal friction force. This has the advantage of being applicable to lengthening as well as shortening. Hill's equation remains of interest, especially because its force-constant  $a$  is obtainable both from mechanical and thermal measurements. Abbott & Lowy

(274) have reported that in *Mytilus* byssus retractor the two values of  $a$  so obtainable are not equal, but this may in time be explained by various peculiarities affecting the experimentation with this muscle.

We continue to regard the force-velocity relation as an expression of the most fundamental kinetic aspects of the contractile structure and refer to attempts (p. 537) to connect this with molecular interpretations.

*The active state.*—The intensity of the active state at a given moment has been defined as the tension exerted when the contractile element neither lengthens nor shortens, or the tension exerted by the contractile elements if this could be directly measured without the series elasticity intervening. At the plateau of full isometric activity, this is equal to the tetanic tension, and it can be approached experimentally in a twitch by the expedient of a rapid stretch [Hill (232, 233)]. Otherwise, tension development in an isometric twitch lags behind the course of the active state, because this becomes externally evident only after the series-elastic elements have been stretched to the same stress, a process requiring time because of the kinetics of shortening of the contractile component. We shall turn to the various experimental methods which have been brought to bear upon this problem.

The rapid stretch method has been alluded to. Its speed of stretch is more critical than was previously evident. Abbott & Mommaerts (224) found for papillary muscle and also for the frog sartorius that a very rapid extension, e.g., during 1 msec., destroys the active state but permits immediate redevelopment in a manner reminiscent of the Ritchie method (see below). In unpublished experiments, furthermore, it was found that even suitable stretches do not in all cases lead to a tension equal to tetanic tension; this may be related to the possibility discussed by Jewell & Wilkie (223) that the full level of the active state may not always be reached in a single twitch. Duration and decline of the active state are investigated most explicitly according to Ritchie (234): at various moments after stimulation, the muscle is released so that its tension drops to zero and returns to an extent determined by the remaining time; one obtains a family of redevelopment curves, the peaks of which are points on the decay curve of the active state. Other approaches have also been informative, but less explicit. The development of the active state has been investigated less, but Sandow (290) has noticed a small break in the derivative of the tension development curve, considered as indicating the end of the onset period. A very direct method has been proposed by Close (235): when a second twitch is initiated during the relaxation from a previous one, the resulting minimum in the compound tension curve is a point on the active state development curve of the second twitch. Close finds that activity starts at about 10 msec. after stimulation and is fully developed at about 40 msec. (at 0°C.), in accordance with the Hill school. His valuable work describes several features of the onset of activity, but we would like to see more explicit evidence for his conclusion that during the phase of partial activation the shortening velocity at zero load already has its full value. A family of curves with different  $V_0$  intercepts was found by



Jewell & Wilkie (270) for the period of decline of the active state in relaxation; admittedly, however, relaxation does not necessarily reverse the sequence occurring in activation.

Instead of these various procedures, Reichel & Bleichert (236 to 240) use the "additional activation" obtainable after a small release as a measure of the activity at the moment of release. The conclusions so obtained are at variance with those of the Hill school. We have not arrived at a complete evaluation of these views, partly because there is no evidence presented that the variation of the extent of release, about 1 per cent of the muscle length, would not influence the course of the activation curve. We disagree with the underlying assumption that such measurements are validly made on slow, and only on slow, muscles; rather, we believe that the various kinetic factors vary more or less in parallel muscles of different speeds [e.g., Fig. 5 of Abbott & Lowy (241)]. One serious problem is raised in any case: in the Ritchie procedure, the tension drops to zero and redevelops to a point on the active state decay curve. Hence, no tension peak should be expected at a given time in excess of a value on this curve, yet such maxima are found by Reichel & Bleichert. Jewell & Wilkie (270) have also commented that there is an element of arbitrariness in the Ritchie procedure, the large releases involved leading to an underestimation of the active state period. According to Goodall (292), the amount of release should have an effect, and he defines an "activated state" in terms of his kinetic concepts. A critical study comparing the different approaches and their underlying assumptions is important; pending this, we shall adhere to the terminology of the Hill school.

*Variations in the active state.*—With experimental methods for the measurement of active state characteristics available, the investigation of their variations has become an important part of muscle physiology. Originally, the British school tended to regard the duration of the active state as the parameter most affected by physiological and pharmacological variations [see Wilkie (221) for early references]. The main case in point was the strengthening of the sartorius twitch by replacing chloride in the medium with nitrate (or iodide or thiocyanate), discovered by Kahn & Sandow (242), shown by Hill & Macpherson (243) to be caused by a lengthening of the active state interval. This has been further studied by Lubin (244) who found a fourfold extension of the plateau duration, without alteration of the tetanic tension or the force-velocity relation, giving more time for a greater fractional development of the potential tension  $P_0$  (mechanism *A*). However, this is not the only possible mechanism. An increased twitch tension at constant duration of the active state could also result from an intensification of the active state (mechanism *B*), and also from a greater intrinsic contraction velocity whereby in the same time a greater fraction of  $P_0$  would be realized (mechanism *C*). Writing the force-velocity equation as  $V = (P_0 - P)b/(P + a)$ , it is seen that both cases *B* and *C* would show a greater contraction velocity, *B* through an increase of  $P_0$ , *C* because of an increased velocity constant  $b$ , assuming independent variability of the several factors. Application of these

principles could elucidate the mechanism of inotropic effects such as those of zinc [Sandow & Isaacson (245)] or of cyclopropane [Sabawala & Dillon (246)]. The lower twitch-tetanus ratio of dystrophic mouse muscle is correlated with a briefer active state, but also with a prolonged relaxation phase and weaker tetanic tension, and is quite complex [Sandow & Brust (247)].

*Inotropic effects in cardiac muscle.*—The mechanisms of variations in contractile strength were explored systematically in the work of Abbott & Mommaerts (224) on the staircase effect and postextrasystolic potentiation of the cat papillary muscle; and in that of Brady *et al.* (248) on the inotropic effects caused by an impulse train applied during the refractory period [or by persistent supramaximal stimulation as discovered by Whalen (249); cf. Furchgott *et al.* (250)], the latter being caused by the stimulation of epinephrine or norepinephrine releasing nerve fibers. A variety of effects were noticed, but in all cases the major strengthening was accomplished without primary alteration of the twitch duration but through a concomitant increase in contraction velocity, which was also separately demonstrated as a shift of the force-velocity curve. Hence, the major inotropic changes are caused either by mechanism *B* or *C*; which one is as yet undecided. Secondary effects, however, such as occur in the development of a staircase of the impulse-train effect, cause passing phases of shortening and lengthening of the active state, and the results of these (by mechanism *A*) are superimposed upon the major inotropic change. Some active state duration measurements on heart muscle were also done by Trendelenburg & Lüllmann (251), whereas several of our viewpoints have been anticipated in the work of Niedergerke (252) in which, however, the relevant dynamic characteristics were not experimentally investigated.

The fact that the intrinsic contraction velocity in the myocardium is greatly variable is in contrast to the situation in skeletal muscle where the force-velocity relation seems to have been regarded as a constant characteristic [Hill (253)]. The contraction velocity was found to increase at higher stimulation frequency; since a force-velocity curve and a force-efficiency curve can be transposed into a velocity-efficiency curve, these data suggest that under conditions of higher cardiac output and heart rate, the heart would have its optimal efficiency at a greater contraction velocity [Mommaerts *et al.* (254)]. Hill (253) has raised the following problem:

If the heart were an ordinary skeletal muscle, and if its efficiency were near the maximum under conditions of severe effort, it would be rather low under conditions of rest or moderate exercise. If its efficiency were near its maximum with moderate exercise, it would be low with maximum effort . . . the question suggested is whether the heart, unlike the skeletal muscle, becomes intrinsically quicker when the frequency of its beat rises.

Our findings seem to have proven this, although the validity of this and related concepts in actual hemodynamic conditions, physiological and pathological, remains to be investigated.

*Energetics of contraction.*—We shall first discuss this from a chemical

viewpoint, continuing the argument that, whatever the primary event in a single contraction may be, in prolonged anaerobic activity of an iodoacetate-poisoned muscle the total energy metabolism takes the form of a splitting of phosphorylcreatine, and that in most frog muscles with which we have experience this metabolism is at 0°C. not accompanied by significant esterification which would cause additional phosphorylcreatine breakdown, unrelated to mechanical activity, in the formation of fructose diphosphate. In such experiments, then, the total energy mobilization ought to correspond quantitatively to the phosphorylcreatine splitting, if we assume that any existing primary reaction would be minor in extent or would be continually reversed by phosphorylcreatine breakdown in the stationary state. This difficult experimental problem has for years been pursued independently by Carlson and by Mommaerts and Seraydarian, and full publication may be anticipated. Originally, the latter authors found the phosphorylcreatine metabolism to increase with work and with shortening, as if the biochemical counterparts of the activation heat, the work, and the shortening heat had been discovered; but recently these authors found, as had Carlson all along, that in some cases upon extrapolation to maximal shortening with zero work, no more (or even less) metabolism is found than isometrically, as if the shortening heat were not accounted for. The former result seemed to agree with the shortening heat as investigated in Hill's classical paper (255), but this presentation takes no account of the effect of the remaining length upon the activation or maintenance heat (see below). This factor might compensate or overcompensate for the shortening heat, as is suggested by Figure 2 of Fenn (256), extrapolating the curve "heat above isometric" to zero load; by Figure 6 of Brown (257); and by recent work of Tigyí (258) who did calorimetric measurements on isometrically contracting versus unloaded muscles and found less cumulative heat produced in the latter.

A monumental work on the energetics of contraction is that by Aubert (259) to which we refer several times in this review. Here, we mention his work on the evolution of the maintenance heat in a tetanus, the rate of which follows a course described as  $h = h_A e^{-at} + h_B$ , this being the quantitative expression of the relatively high initial heat production which in the course of seconds approaches a stable rate  $h_B$ . These quantities depend on the length of the muscle, most clearly as a function of the actively developed tension  $P_0$ . Below resting length  $l_0$ , both  $h_A$  and  $h_B$  vary about equally,  $h_B$  being strictly linear with  $P_0$ , but with a finite intercept for  $P_0 = \text{zero}$ . Above  $l_0$ ,  $h_A$  is fairly constant, but  $h_B$  varies again linearly, but with a different proportionality factor and extrapolating to zero for  $P_0 = \text{zero}$ . In single twitches, Hill (261) also finds a constant relation between developed force and liberated energy, each diminishing with length. This dependence of the maintenance heat upon length is evidently important in relation to the chemical studies by Carlson and ourselves referred to above.

Aubert also finds that in a tetanus, up to 20 stimuli per second at 0°, the maintenance heat is pulsed even though the mechanogram is fused [recalling

the work of Nicolai (260), showing optically a rhythmic activity of the sarcomeres in such a condition].

A most significant observation, first reported by Hill (255), then by Abbott *et al.* (262), and now experimentally improved by Hill & Howarth (263) and Hill (264), is that work done upon a contracting frog sartorius by stretching it does not appear as heat, but is absorbed otherwise, presumably by reversing or preventing metabolic reactions. This absorption is currently being investigated biochemically in our laboratory.

Maréchal & Aubert (265) describe the fatigue in the course of a tetanus of the frog sartorius at 18°C. as having two phases. In the first few seconds, tension drops slowly and heat production is not altered; after a 1-sec. interruption, it resumes its identical course. After about 6 sec., tension drops rapidly and thermogenesis with it, interpreted as an elimination of contractile elements; a brief interruption causes immediate recovery of the contractile force. Some of the early tension loss may be ascribable to internal stretch of elastic elements, since a small extension sometimes restores the original force [Maréchal & Aubert (266); Maréchal (267)]. In a fatigue series, mechanical and electrical activity sometimes decrease together [Grieve (268)], but not always [Bergmans & Maréchal (268a), Seraydarian & Abbott, unpublished], showing that fatigue can have several causes, even in isolated muscle.

*Relaxation.*—Compared to the dynamics of contraction, that of relaxation has received little attention. In contradiction to certain earlier views, Abbott & Lowy (269) demonstrated that the time course of relaxation differs from that of stress-relaxation after a stretch. Aubert (259) has shown that most of the course of relaxation, as well as of the associated heat liberation, can be represented by a probability curve, as if there were a random inactivation progress of independent contractile entities, as in earlier proposals by Buchthal's school. A recent study on relaxation is that of Jewell & Wilkie (270), with the starting point that the duration of relaxation in an isometric contraction is longer than that of an isotonic one on a comparable scale, showing the influence of length changes upon the relaxation process. During relaxation, "activity" seems to persist longer than indicated by the Ritchie method. Under a variety of conditions, the time constant of the major decay process is remarkably constant, as if it were governed by a fundamental kinetic process.

*Lamelibranch smooth muscle.*—Because of the heterogeneity of smooth muscle [cf. Mommaerts (271)], it is doubtful whether any special investigation can be regarded as "typical" for the group as a whole. We wish to give special prominence to investigations on lamelibranch smooth muscles, especially on the anterior byssus retractor muscle of *Mytilus*. This consists of fibers running the full length of the muscle, which has facilitated the description of both the bioelectric and mechanical properties. As described in 1937 in the classical papers by Winton and by Fletcher, this muscle shows two types of contractions. The phasic response occurs as minute single contractions upon single shocks, or as tetanic responses upon alternating or

repetitive stimuli (e.g., at 10 per sec.), followed by relaxation over a time less than a minute. The tonic response is elicited at the cathode by direct current stimulation and is maintained without action potentials for many minutes, and can at any time be interrupted by an alternating current stimulus. Twarog (272) demonstrated that a tonic response is also obtained during the application of acetylcholine, that it persists after washout of the reagent which causes a reversal of the depolarization, and that instant relaxation of this contraction is caused by 5-hydroxytryptamine. The latter also inhibits the electrically caused tonus, and acetylcholine tonus is also reversed by alternating current stimulation. These various facts are ascribed to the occurrence of multiple cholinergic and serotonergic nerve elements, and further electrophysiological data on their differential stimulation are given by Cambridge *et al.* (273).

Abbott & Lowy (274) showed that the phasic contraction follows the force-velocity equation of Hill with different constants, the shortening speed being about 25 times that in the frog sartorius, and relaxation being proportionally slower still. A maintained tetanus begins to decline after about 30 sec. at 15°C.; if this were regarded as fatigue, it would further accentuate the difference from tonic contraction which persists so much longer. The active state declines 10 to 100 times faster than the tension [Lowy & Millman (275)]; in tonic contraction, tension outlasts the active state even more [Lowy & Millman (276)]. The muscle shows no all-or-none behavior, but displays decremental conduction, summation, and facilitation [Schmandt & Sleator (277)]. Some of these properties differ from those of the foot retractor muscle, which is faster, responds with a larger twitch, and shows summation but no facilitation [Abbott & Lowy (274)]. There is a low maintenance heat in tetanus (that, if any, in tonus has not yet been recorded), and there is a shortening heat.

In a phasic response there is fairly complete tension recovery after a release, with some difference between the results of Abbott & Lowy (274) and those of Jewell (278). There is also complete release-recovery during a DC stimulus and in the presence of acetylcholine, but during the period of maintenance of tonic tension after cessation of this stimulus or after removal of acetylcholine, recovery is nearly abolished. By the use of the Taylor (279) electrode system, Johnson & Twarog (280) have greatly advanced the study of electrically induced tonus and find no "active state" during maintenance of tonic tension.

One arrives at the concept (the peculiarities of the multiple innervation determining the effects of the different forms of stimulation) that the excited muscle shows an active phasic response not unlike that of skeletal muscle, but that in the tonic condition the muscle is "frozen in" at the prevailing state of shortening or tension by a "catch" or "ratchet" mechanism [see notably Jewell (278)]. Even skeletal muscle gives a faint indication of such behavior, as suggested by the well-known improvement of the economy of tension maintenance in the course of a tetanus [cf. Aubert (259)], or by some

observations of Jewell & Wilkie (270) on the slowing of relaxation after a prolonged tetanus, and Maréchal (280a) has discussed the similarities between a skeletal muscle in iodoacetate contracture and smooth muscle in tonus. The full and extreme development of this feature in the anterior byssus retractor muscle and other smooth muscle indicates the participation of a specialized mechanism. We have already mentioned the presumed role of paramyosin (tropomyosin A) in that regard and now give the following physiological demonstrations. According to Lowy & Millman (281), the stress relaxation of the anterior byssus retractor muscle shows three phases instead of the usual two; the slow one is typical for paramyosin muscle and is abolished by 5-hydroxytryptamine. Johnson *et al.* (282) observed that the shortening of a glycerol-extracted anterior byssus retractor muscle with ATP is prevented in electrolyte media causing the crystallization of paramyosin, while isometric tension development is not so affected. This suggests clearly that structurally bonded paramyosin interferes with length changes brought about previously. By implication, active contraction is then ascribed to the actomyosin structures. While these views are very plausible, there are unexplained points, and the very high contractile strength, up to 11 kg. per cm.<sup>2</sup> [Jewell (278)], is hard to explain if it is caused entirely by the relatively small amount of actomyosin, if we take vertebrate muscle as a measure of comparison.

*Physical phenomena accompanying activity.*—Ernst and his group (283) made further studies on the volume constriction of muscle applying a piezo-electric method. They express the results of these and other investigations in terms of myosin crystallization (284, 285). A variable capacitance dilatometer has been developed in this laboratory, with which Abbott & Baskin (286) record an early volume increase, preceding the diminution found by Ernst. This expansion has the same time course as the latency relaxation and is affected by the initial length of the muscle in a different way from the constriction effect. The time course of the constriction suggests its correlation with the active state, and unpublished experiments with the Taylor (279) electrode show that the volume decrease persists in continued depolarization.

Goodall (287) studied the effect of massive-electrode stimulus strength upon latency-period phenomena in turtle muscles. With increasing field strength, these three effects occur in sequence: a decrease of the latency period, an increase of the initial rate of tension development, and the appearance of latency relaxation.

*Quantitative interpretation of the course of a contraction.*—We make brief mention of several contributions which are not "theories" in the sense of explaining mechanisms, but which attempt to derive the time course of a twitch from the force-velocity relation, the extensibility of the elastic component, and other relevant quantities. One such calculation [Ritchie & Wilkie (288)] gives a fair account of the contraction phase of a frog sartorius twitch, based upon the Hill equation, while Carlson (289) refrains from expressing



the force-velocity relation algebraically. Sandow (290), along generally similar lines, introduces a time-dependent exponential approach for the maximal tension to its final value; this accounts notably for the sigmoid start of tension development. Finally, Goodall (291, 292) presents a kinetic treatment distinguished by including the effect of length change upon activation, which introduced a feedback term permitting the occurrence of oscillations. This is of particular significance for problems in insect flight (293, 294).

*Theories of the mechanism of contraction.*—Fortunately or unfortunately, there has not been a great harvest of theories on the mechanism of contraction during the review period. For some years attempts at theories have been influenced by the possibilities created by the understanding of the coiling behavior of macromolecules (171). The plausibility of a sliding filament model necessitated a search for mechanochemical models to explain such an event, the first one being contributed by Weber (199).

Two theoretical contributions must be regarded with special interest. The first of these, by Huxley (295), aims directly at a sliding filament model. Myosin and actin filaments lie close together, the latter carrying groups A that can combine with groups M on the myosin which are placed on a thermal oscillator. Combined with M, A is carried over a distance until dissociated from A by a reaction, say the actomyosin dissociation by ATP. The likelihood of this detachment is described by a rate constant  $g$  which is small when approaching an equilibrium position 0 of the oscillator, whereupon it jumps to its maximal value, assuring unidirectional motion. We have considered whether this assumption of an asymmetric reaction probability does not contain a Maxwell demon in sheep's clothes because while the entire process proceeds at the expenditure of chemical energy, a proposed scheme may be in accordance with thermodynamic bookkeeping and yet contain inadmissible steps. We do not believe, however, that this objection is valid here. Although the proposed pendulum-model may be arbitrary, fundamentally this type of mechanism falls in line with Koshland's (128) ideas of the effect upon reaction rate of configurational changes near the active site of an enzyme. The mathematical formulation of Huxley's theory permits the calculation of several dynamic characteristics of active muscle.

The other theoretical contribution is that by Podolsky (296, 297), further elaborations of which may be expected in relation to current experimental work by that author. As far as published, its main contribution is to present energetics and to specify relations between various dynamic factors so as to introduce a control of chemical reaction rate by shortening.

#### EXCITATION AND ITS LINK TO CONTRACTION

*Membrane theories and alternative views.*—A full presentation of these matters, beyond the scope of this review, has been given with great authority by Hodgkin (298, 333), Shanes (299, 300), and Glynn (301). However, we must refer to persisting discussions, also affecting muscle physiology, on the basis of ion distribution and excitability. The predominant views are those



expressed in Goldman's constant field theory and its elaboration by Hodgkin & Katz, involving the following assumptions. (a) In the membrane, the mobility of ions under the influence of electrical and concentration fields is similar to that in free solution, except for permeability factors  $P_i$  which are constant in a given state, but alterable in excitation or otherwise. (b) The potential gradient is constant throughout the membrane transversely and is zero longitudinally when not excited. (c) The membrane is homogeneous (dependent, of course, on the scale of magnitude one wants to consider), implying that both on its inside and outside surface the same partition function describes the ionic concentration at the interface as a function of that in the solution. (d) Such partition functions determine the ionic concentration in the surface dependent on those in free solutions, and an average intracellular ionic concentration can be chosen to represent the ionic concentration near the inside of the membrane. While all these assumptions are reasonable, they are not equally evident, and the latter especially has been the subject of alternate views of greater or less deviation. Thus, Edwards & Harris (302) ascribe a considerable part of the slower exchanging sodium of whole muscle to an outer, probably intracellular, depot, where it is held by chemically labile groups, with potassium in an internal phase, not bound, but restricted in its diffusion because of the anionic elements of the protoplasm. Ernst (303) and Tigyí (304) regard some intracellular ions as bound and not easily exchangeable, while Shaw *et al.* (305) suggest that the muscle cell consists of three compartments, extracellular, bound intracellular, and free intracellular, the latter determining the ionic activity at the membrane. The binding is ascribed to a lattice-ordered structure, occupying two-thirds of the cell volume.

Dr. L. B. Nanninga (unpublished) is attempting to compute the free potassium on the basis of the known potassium and magnesium affinities of several components; the major ones considered so far, myosin and ATP, seem to permit no more than 5 per cent or so of the potassium to be bound. Also, the measurements by Lewis & Saroff (306) show that myosin binds sodium more than potassium, while Fenn (307) made the corresponding observations on a glycerol-extracted fiber preparation. Such findings would eliminate theories like the early fixed-charge hypothesis of Ling (308); its newer formulation (309), as well as the proposals of Troschin (310), seeks to avoid such difficulties by assigning special binding properties to complex colloidal structures. This issue has been competently and critically discussed by Booiij (311), who lists objections to views of this type, while proposing ideas as to the role and nature of membranes. Even if such views should not become generally accepted for the protoplasm as a whole, they might contribute greatly to the understanding of the architecture of the membrane itself, where one eventually will have to account for such crucial properties as variable permeabilities for individual ions in terms of molecular architecture and its alterations. A chemical result of importance in this connection is that sodium and potassium differ in highly specific interactions with

polyvinylsulfonic acid (312) and with carrageenin (313), although there is no evidence of selective binding affinity.

*Ion exchange phenomena.*—The evaluation of the size of the extracellular space remains as difficult as it is essential. Tasker *et al.* (314), comparing the distribution of several substances, find this space (about 22 per cent) to differ: sucrose space > inulin space > albumin space, suggesting inverse proportionality to the size of the marker molecule. They also find that eventually both sucrose and inulin penetrate into the cells and that the extracellular space in individual toad sartorius muscles may vary between 8 and 40 per cent. For unblotted muscle, Adrian (315) finds the chloride space (measured with  $\text{Cl}^{36}$ ) to be 29 per cent, considerably above the usual estimate.

Studies by Keynes & Swan (316, 317) and Simon *et al.* (318) showed that lithium replaces sodium to a large extent, the resting and action potentials not being changed by more than 2 mv. after 20 min. in Li-Ringer. From such a medium, there is a Li influx of 1 to 2 pmole  $\text{cm}^{-2} \text{sec}^{-1}$ , roughly equivalent to the non-exchange-diffusion fraction of Na flux (319, 320). Lithium is thought to replace Na in the passive Na channels but any active extrusion of Li is 10 or more times slower than Na.

Replacing external Na with Li or choline halves the Na-efflux (316, 317): a Na-Na exchange diffusion (319) accounts for half the Na exchange. After 12 hr. soaking in Ringer, or 3 hr. in K-free Ringer, reduction of external Na does not alter Na efflux. The absence of the Na-Na exchange in this condition is attributed to an increase in internal Na, since it becomes re-established after keeping in Li-Ringer. Neither a single nor a two-compartment theory for the internal Na accounted for all the major findings. As to the possibility of a large intermediate store of Na [Carey & Conway (321)], Hodgkin & Horowitz (322) point out that this must exchange within 2 min. of washout in an isolated muscle fiber, because no such separate fraction was noticed in their experiments, a single exponential curve being obtained for both Na uptake and efflux from the first point of observation on. Edwards & Harris (302) believe that much of the Na washout comes from an outer but still intracellular region. There, it is held by "chemically labile groups" because Na efflux depends on external K as does metabolism, and for other reasons. The extrusion from Na-loaded muscles is not altered by cyanide and is in excess of that accounted for by  $\text{O}_2$  consumption [Frazier & Keynes (323)].

In a single fiber, Hodgkin & Horowitz (322) find that both influx and efflux of potassium obey a single exponential law of the type expected for a membrane-limited diffusion. As an explanation of opposite results of Edwards & Harris (302), these authors point out that in whole muscle, variations in fiber diameter and of exchange constants of the membrane and other factors may complicate the situation [Creese *et al.* (324)]. Replacement of Cl by other anions has little effect upon the Na and K exchanges (325).

The relative influxes of several cations are Na:K:Rb:Ca = 0.043:1:0.54:0.1 [Mullins (326)]. Almost all K can be replaced by Cs or Rb without an increase in intracellular Na [Lubin & Schneider (327)]. Divalent cation

fluxes are over 100 times slower ( $\text{Ca}:\text{Ba}:\text{Ra}=1:3:1$ ), and Ba and Ca uptakes were greater when the muscles were spontaneously active [Mullins & Moore (328)].

Efflux of  $\text{Cl}^{36}$  is exponential from KCl-loaded muscles when the membrane apparently behaves like a Cl electrode (see above) but is continuously flattening from NaCl-loaded muscles whose K conductance is appreciable [Harris (329)]. The pH inside large crab muscle fibers has been measured directly with a micro-glass electrode by Caldwell (330).

*The resting potential.*—The argument has been used that the resting potential is less than the theoretical K potential and therefore is not a Donnan potential; a limited coupling between K efflux and Na influx might account for the discrepancy (331). On the other hand, Kernan (332) finds agreement between the theoretical and the measured potential in muscles perfused with plasma. It might be that in the absence of a plasma constituent, a coupled Na-K exchange arises. By analogy, the squid axon *in situ* shows only a small negative after potential compared to an excised axon in sea water (298), the latter having a coupled Na-K exchange. It is likely that both ion permeabilities and ion exchange mechanisms are altered in artificial solutions.

Hodgkin & Horowicz (333) showed that the resting potential of single muscle fibers depends on both K and Cl. It behaves as a K electrode when K and Cl are varied reciprocally, or if external K is changed in a Cl-free solution. At constant external K, changes in Cl produce changes as for a Cl-electrode, but transiently for 1 to 60 min. At constant Cl, changing the external K from 2.5 to 10 mM produces a sudden depolarization, followed by a drift towards a new steady value; upon return to 2.5 mM, only a small depolarization occurs immediately, an hour being required to obtain complete return of the original. These and other results are explicable by a scheme in which K and Cl concentration potentials are in parallel, but the K system contains a rectifier such that its conductance inward is much higher than outward; in addition, the outward conductance is inversely proportional to the driving force on K, i.e., its concentration gradient and the membrane potential. In short, the potential depends on  $V_K$  if  $V_K > V_{Cl}$ , and on  $V_{Cl}$  for the opposite case. For a muscle fiber in normal Ringer, the inward K permeability was calculated as  $8 \times 10^{-6}$  cm. sec.<sup>-1</sup> and the outward only  $0.05 \times 10^{-6}$  cm. sec.<sup>-1</sup>. Chloride permeability, on the other hand, remained constant at  $4 \times 10^{-4}$  cm. sec.<sup>-1</sup> in both directions. By chemical determination of internal concentrations, Adrian (315) followed the movements of KCl in and out of the cell, and finds also that inward movement is much more rapid than outward movement. Fibers equilibrated in 100 mM KCl will show a resting potential of +60 mv. in Cl-free Ringer, slowly falling, with oscillations, to a stable -20 mv. An inverted action potential could sometimes be elicited (see Falk, *in press*, for further observations on oscillatory behavior).

By replacing Cl with other substances, Hutter & Noble (334) [cf. Frank (335)] showed that Cl-conductance accounts for 68 per cent of the resting

membrane conductance, in agreement with the 2:1 ratio for Cl:K conductances found by Hodgkin & Horowicz (333); K conductance increased in an electrically hyperpolarized membrane, but Cl-conductance did not change. In the cold, the action potential was prolonged in Cl-free Ringer, indicating that Cl carries appreciable current during repolarization; at room temperature, only the after potential was affected. Hutter & Noble suggest that changes in membrane resistance may produce changes in mechanical behavior by altering the membrane potential. By replacement of Cl with other anions, Hutter & Padsha (336) found the following membrane resistance ratios: Cl:Br:NO<sub>3</sub>:I = 1:1.5:2:2.3. Certainly, Br, NO<sub>3</sub>, and presumably I, will slightly permeate the membrane, although the demonstration of the latter occurrence by Simon *et al.* (318) suffers because the washout curve is continuously flattening. The effect of CNS is more complex.

Fibers soaked for 1 to 18 hr. in K-free Ringer lose one-third or more of their intracellular K, and the resting potential falls to -40 mv., remaining there after a large measure of restoration of the cellular K [Stephenson (337)]. Would the membrane potential now be Cl-dependent? After such partial recovery, there was 70 mM internal Na, indicating that both K and Na permeability may be elevated. The fibers were still excitable. Stephenson believes his results indicate that the membrane potential is not primarily a diffusion potential. Eckel *et al.* (338) made the interesting discovery that in K-deficient rats, intracellular K is partly replaced by the diamino acid, L-lysine. Electrophysiological studies on this material should be of interest.

Lüllmann (339) concluded that the failure of the resting potential of rat diaphragm to change during intracellular K loss after denervation would require a concomitant fall in K permeability in the Goldman equation. Ware *et al.* (340) found the resting potential of mouse tibialis to fall from 100 to 80 mv. *in vivo*, three to five days after denervation, only after the onset of fibrillation. These two sets of data, in conjunction with discussions on K and Cl conductions, are consistent with the view that denervation shifts the Cl:K conductance ratio in favor of Cl, and that prolonged fibrillation elevates internal Na and Cl, and reduces potassium.

The depolarization by Rb and Cs is accounted for by the constant field theory by making allowance for a concentration dependence of the permeability coefficients [Sjodin (341)]. Thallium, with a crystal radius between K and Rb, distributed across the membrane like potassium. External Tl causes depolarization, by about 58 mv. per decade concentration increase; stimulation causes a 300-fold increase in the Tl efflux; the membrane seems unable to distinguish between Tl and K [Mullins & Moore (328)].

*The action potential.*—A considerable part of the ionic hypothesis of electrical activity proposed for nerve has been found applicable to skeletal muscle. Thus, Hodgkin & Horowicz (322) determined a net Na entry of 15.6 and a net K exit of 9.6 pmoles cm.<sup>-2</sup> impulse<sup>-1</sup> for single frog muscle fibers; a Na influx of this order is estimated as the minimum for depolarization. Creese *et al.* (342) and Voronova (343) also report an increase in Na uptake

and K loss in active muscle. The rapidity of repolarization indicates that the K conductance must increase during the later part of the spike. Håkansson (344) compared the externally recorded action potential with the transmembrane potential, finding that the extracellular current is proportional to the second time derivative of the membrane potential and that the total charge flow is  $1.4 \times 10^{-6}$  coulombs  $\text{cm}^{-2}$ , corresponding to 14 pmoles  $\text{cm}^{-2}$  impulse $^{-1}$  of monovalent ions, in remarkable agreement with the tracer values of Hodgkin & Horowicz. The membrane potential displacements in muscle in response to subthreshold current pulses were found by Jenerick (345) to follow the core-conductor error function both in time and space, up to the activation threshold for Na which is reached at 55 to 60 mv. In Na-free media a K activation potential was observed near 35 mv. A tenfold increase in Ca lowered the K and especially the Na activation voltages; these two voltages are then close together, requiring a rapidly rising stimulus to excite the membrane so that the threshold is raised and accommodation increased. All these results are strikingly similar to those obtained on the squid axon. Calcium also hyperpolarized the membrane slightly [Jenerick (346)], with the corresponding increase in maximum depolarization and overshoot. While the rate constant for the rising phase increases with decreasing Ca, the falling phase is unaffected; the negative after-potential is elevated by about 50 per cent for a tenfold increase in calcium.

The direct effect of Cl movement in the action potential was measured by Hutter & Noble (334), showing that replacement of Cl by methylsulfate markedly slows repolarization at low temperature, while at 20°C. it only causes an increase in size and duration of the after-potential; this lack of influence at the higher temperature is ascribed to a postulated high temperature coefficient for the increase in K conductance.

The after-potential is held to be linked with energy metabolism because of its high  $Q_{10}$  below 10°C. and its alteration by metabolic inhibitors [Macfarlane & Meares (347, 348)]. Hutter & Noble (334) believe that this dependence need not be direct because, according to Benoit *et al.* (349) and Desmedt (350), the transition between spike and the after-potential occurs at a fairly constant potential level under a wide variety of conditions, while the resting potential is temperature sensitive. We personally believe that the often cited relation between the after-potential and metabolism is too unspecific to be of much value.

Some membrane constants of cat tenuissimus at 37° have been determined by Boyd & Martin (351) as follows, with comparison for values in the frog at 0° in brackets [Fatt & Katz (352)]:  $R_m = 1450$  ohms  $\text{cm}^2$  (1400);  $C_m = 2$  to 5  $\mu\text{f. cm}^{-2}$  (6-8);  $Q_{10}$  of  $R_i = 1.3$  (4);  $Q_{10}$  of  $R_m = +2$  (-1.35), [del Castillo & Machne (353)]. Conduction velocity in the human vastus externus in voluntary contraction is 4.26 m.sec $^{-1}$  [Meda & Ferroni (354)] as compared to 2.2 m.sec $^{-1}$  in the frog [Håkansson (344)]. The only effect of stretch on membrane properties in the toad sartorius is a small decrease in resting potential and negative after-potential [Ishiko (355)]. Tonic muscle

fibers of the frog have a resting potential of only 61 mv., not explained by the intracellular K concentration [Kiessling (356)].

The behavior of crab muscle has been further studied by Fatt & Ginsborg (357) showing that Na can be replaced by Sr, a resting potential of 90 mv. and action potential of 135 mv. being recorded; similarly, excitability was maintained in 160 mM Ba, but here the action potential was prolonged. A measured increase in membrane conductance during the action potential proportional to the external Sr, and a 100-fold increase in total membrane conductance suggest that the action potential results from a high permeability to these ions. With prolonged soaking in tetraethylammonium salt, action potentials could be elicited in media with Ca as the only cation; this is attributed to an irreversible alteration of the membrane permeability. Glutamate causes a contraction of crustacean muscle, followed by a refractoriness toward indirect but not direct stimulation. The glutamate contraction is depressed by stimulation of the inhibitory axon [van Harreveld (358, 359)];  $\gamma$ -aminobutyrate antagonized the glutamate effect, whereas earlier investigation had shown this substance to inhibit muscular activity. The chloride permeability of the membrane is increased by  $\gamma$ -aminobutyrate, as it is by inhibitory stimulation [Boistel & Fatt (360)].

*Excitation of smooth muscle.*—The stimulation of smooth muscle and the propagation of impulses in smooth muscle organs are problems of great variety and complexity. However, since the introduction of intracellular recording of smooth muscle by Bülbring, certain advances in the understanding of mammalian plain muscles have been made. In longitudinal intestine muscle, there is a proportionality between tension and spike frequency; epinephrine or anodal polarization decrease and acetylcholine or cathodal polarization increase the discharge rate and the tension correspondingly [Burnstock (361, 362)]. Holman (363), working with higher-resistance electrodes, gives the following characteristics: a membrane potential of 51.5 mv., an action potential overshoot of 10 mv., a prepotential preceding the spike, and a dependence of the membrane potential upon the potassium concentration up to 30 mM or more. A modification of the sucrose-gap technique has been applied for a study of electrophysiological time characteristics [Burnstock & Straub (364)]. Bülbring *et al.* (365) found that a single shock would not affect a spontaneously active preparation, but a tetanic stimulation would interrupt rhythmicity and cause a regular response; when inactive, the tissue would also respond to a single shock.

In the proboscis retractor of *Phascolosoma* (Golfingia), conduction is entirely by nerves [Prosser & Sperelakis (366)]; there seems to be a double innervation for fast and slow contractions; conduction persists when the muscle is fatigued, and transmission can be blocked by tetracaine, not by curarine. Another conduction mechanism is by mechanical pull, since many smooth muscles respond actively to stretch. This occurs in the intestinal retractor of Golfingia [Prosser *et al.* (367)] and perhaps in uterine muscle [West & Landa (368)], but not in others such as dogfish mesentery, Golfingia



proboscis retractor, or in the circular muscle of cat intestine [Sperelakis & Prosser (369)]. In the latter case, and in the guinea pig taenia coli [Bülbring *et al.* (365)], ephaptic transmission seems the most likely mechanism. Descriptions have appeared of protoplasmic linkages between cells in ureter [Bergman (370)] and in rat intestine [Thaemert (371)]; the latter author regards the protoplasmic contacts as transitory. While one will generally assume that the membrane current is the initiating feature of excitation, Singh & Acharya (372) find excitation in the frog stomach in the absence of an ionic gradient.

*Uncoupling of contraction from excitation.*—In the early stages of exploration of a new field, it is often useful to learn how the connections between various phenomena can be interrupted. Therefore, we shall consider examples of disconnecting contraction from excitation. When a frog muscle is immersed in Ringer which is  $2\frac{1}{2}$ -fold hypertonic, contraction ceases but impulse conduction remains (373, 374). In that condition, stimulation still causes heat production to about the amount estimated for the activation heat (375). It would appear, therefore, as if in this treatment one had maintained the link between excitation and energy release, but uncoupled that between energy release and contraction. However, there is a response to tetanic stimulation; it is merely that contraction velocity has become extremely low [Howarth (376)] and this may be a direct consequence of dehydration. Further work is needed to decide just what significance these interesting observations will assume. Meanwhile, they have served as a clear illustration of the priority of heat production over contraction [Hill (377)]. A second case of uncoupling is that caused by dinitrophenol [Kuschinsky *et al.* (378)], which may lend itself to further analysis. Dinitrophenol also causes such dissociation in intestinal smooth muscle, as does azide [Bülbring & Lüllmann (379)]; the tissue relaxes fully, while spike frequency and oxygen consumption increase.

In a different category are those studies in which the link between stimulation and response is varied by alterations in the extracellular electrolyte composition. In principle, all studies on the effect of extracellular ions upon excitation and contraction can contribute to this, and some such examples are mentioned under the next heading.

*The link between excitation and contraction.*—From the point of view of the membrane theory, the connection consists of an activation of the contractile structure by an event of membrane-excitation, the latter usually in the form of a propagated potential. There are of course instances of non-propagated potentials, and the work by Huxley & Taylor shows that, fundamentally, a local response in regular skeletal muscle can activate a limited area of contractile matter adjacent to it. Csapo & Suzuki (380) presented evidence in favor of a modified form of the earlier "window field" theory according to which contraction is caused by the combined action of depolarization and internal current flow, but Sten-Knudsen, working alone (381) and with Buchthal (382) in a careful reinvestigation of the problem, seems to have eliminated this hypothesis definitively. Among their most crucial



experiments is the demonstration that when impulse conduction is blocked, a longitudinal field causes only stimulation of the end region of the cell where the membrane is transected by field lines, while the middle part remains inactive. Conversely, such a muscle can be almost fully activated by transverse stimulation. We shall adhere, therefore, to the more generally held views.

Now, excitation is usually presented in terms of depolarization, and we shall first discuss whether the fact of depolarization by itself might determine the active state to be "on". Certain instances are suggestive of this, e.g., in ventricular cardiac muscle there is a general correlation between the duration of the plateau of depolarization and that of the active state that one might roughly estimate from the twitch duration (Brady, unpublished). Even more striking is that the maintenance of depolarization in cardiac muscle by a capillary electrode arrangement [Kavalier (383)] for as long as 2 sec. causes contraction to be maintained correspondingly. In unpublished experiments, we made similar observations on the sartorius of the frog with the Taylor (279) electrode system. On the other hand, it is obvious that in skeletal muscle the spike potential is normally much shorter than the contraction. The question is not whether the "plateau" in cardiac and the after-potential in skeletal muscle may be considered as equivalent; the point is that there are many cases in which there is active contraction when depolarization is over. Hence, the membrane potential as such is not, without further qualification, the determinant factor, and the other more fundamental attributes of the membrane response must be investigated.

Important information regarding the coupling between excitation and contraction in crustacean muscles is contained in three papers by Hoyle & Wiersma (384 to 386). The first and second deal with the effect of stimulating excitatory and inhibitory nerves. The most relevant part of the study deals with the coupling between membrane potential and contraction. In certain cases, stimulation of an inhibitory fiber during contraction reduces the depolarization (even hyperpolarization may occur), and the contractile strength vanishes correspondingly, as if the two phenomena were coupled; in fact, when the extent of repolarization is varied by changing the frequency of the inhibiting stimulus, the remaining tension varies too, if not exactly in proportion. In other cases, in the so-called paradox state, one finds that contraction ceased in response to stimulation of the fast axon, although the resulting depolarization had not changed, and the mechanical response to stimulation of the slow axon remained normal. Such observations speak against a direct link between membrane potential and contraction. Instead, the action of coupling substances is considered, calcium ions being listed among the possibilities.

When considering the duration of maintenance of the response, the variation of the active state period by nitrate, iodide, or thiocyanate would offer favorable possibilities. Apart from some quantitative disagreements, the after potentials are increased and lengthened in that order (334, 387,

388); there are correlated changes in membrane conductance (334, 336). What other electrochemical correlates are changed? Edwards *et al.* (325) studied the Na and K fluxes under the influence of these anions, but unfortunately only in resting muscle, so that this relevant information is still lacking. The only, but very significant, information comes from Bianchi & Shanes (389): the exchange of isotopic Ca is increased in activity, and this increase is enhanced by the potentiating anions. So we have one important point: the amount of Ca passing through the membrane is correlated with the duration of the fundamental event elicited. In more general terms, evidence for the role of Ca in excitation and coupling had already been accumulating (390 to 392).

A second line of evidence begins to emerge from the work by Brady *et al.* (248) on the potentiation of cardiac muscle by mechanisms effecting a release of epinephrine or norepinephrine. This causes an alteration of the intrinsic contraction velocity and (or) of the intensity of the active state. If the agent acts upon the cell exterior, this alteration would indicate that an unspecified property of the membrane exerts a control over the dynamic characteristics of the contractile structure. Similarly, the unknown factors, which may or may not be membrane properties, operative in the staircase phenomenon act upon those same characteristics. Less completely clarified is the result of Brady *et al.* that the extrastimulation during the refractory period also affects the kinetics of contractility. In this connection, we refer back (p. 85) to the work of Goodall (287) whose results show that if the membrane depolarization is forced to greater amplitude, the kinetics of early mechanical events are considerably modified.

In a different way, the problem is being approached by Hodgkin & Horowicz (392a) by studying the responses to depolarizations induced by suddenly raising the potassium concentration facing a single muscle fiber. This response also requires calcium in the extracellular medium [Frank (392)]. Immersion of a whole muscle into a high potassium medium, without the precautions for the rapidity of the change at the individual cell membranes, may or may not cause a transient phase of twitching, but in any case causes a persistent increase of the metabolism without correlated mechanical activity. This is sometimes known as the Solandt effect, specifically when studied myothermically, as most recently by Hill & Howarth (393). This topic, too, is of great interest in relation to the excitation mechanism, since it represents an activation of metabolism with an uncoupling of the contraction response. The thresholds for the activation of respiration and of glycolysis differ, pointing to several sensitive sites for metabolic regulation, and with respect to glycolysis, Kaye & Mommaerts (30) have found that, again, calcium is a necessary link in the activation process.

*Structural basis of excitation and coupling processes.*—Brief reference is made to important studies on the submicroscopic structure of the myoneural junction (394 to 396).

The role of a structural component in the inward conduction of excitation

has been obvious since Hill calculated that no ordinary diffusion of a substance from the membrane could be fast enough to account for the rapidity of the contractile response. Recent developments have ascribed such a role to a tubular system first studied by Retzius in 1881 and investigated electron-microscopically by Bennett, Porter, Palade, Edwards, and others [see Bennett (206)]. The physiological experiments responsible for this allocation were those of Huxley & Taylor (397) and Huxley (398). In these it was found that isolated fibers display local contractions, extending inward over a limited distance, and lengthwise over one or two sarcomeres, when stimulated by weak pulses (not generating a conducted impulse) from an external microelectrode, only when this electrode is placed on certain critical areas. These could be located as discrete circumferentially located spots at the level of the Z-membrane in the frog, but in lizard and crab muscle they were placed on both sides of this near the A-I boundary. This corresponds to the positions of sets of vesicular structures called triads, as observed by Porter & Palade (399) and Robertson (394). It is assumed, then, that these triads constitute the sites with the lowest stimulation threshold, from which the state of excitation is further distributed by the tubular system of the sarcoplasmic reticulum [cf. Bennett (206)]. Considerable further detail has been added to knowledge of these structures by Andersson-Cedergren (396) for mouse skeletal muscle. She distinguishes three independent entities. The T-system is a set of transverse tubes (like the others of a few hundred Å diameter), extending from the membrane inward at the level of the A-I border, and is not continuous with the membrane and the other tubular components. The I-system branches longitudinally in the interfibrillary space from the T-system toward the Z-line and is continuous with the same system of the neighboring sarcomere. The A-system, conversely, extends along the A-bands. Near the T-system, the A- and I-systems, too, have transversely oriented branches, and these triple sets form the triads of Porter & Palade. One would assume that the T-system is primarily engaged in the inward spread of excitation, while the other components may or may not take part in the longitudinal propagation. The transfer of excitation from the membrane to the tubular system, and between the components of these, would then be of a microsynaptic nature.

An investigation by Natori & Sakai (400) will provide some food for thought: exposed fibrils from frog muscle can be stimulated electrically. Garamvölgyi (401, 401a) finds this also true for isolated fibrils from flight muscles of bees; their contraction can be further enhanced by ATP.

For smooth muscle, less knowledge is available, but Peachey & Porter (402) have attempted to relate the lesser prominence of the reticular system here with the slower response of the contractile system.

In cardiac muscle, according to Porter & Palade (399) and Lindner (403), structures exist which are roughly comparable to those in striated voluntary muscle. A special feature here is introduced by the intercalated discs (404 to 406). These are continuous with the cell membrane and are looked upon as

cell boundaries, so that the heart is not a syncytium, although of course the resistance they offer to the impulse spread cannot be judged from structural investigations.

The electronmicroscopic structure of the Purkinje fibers has been studied by Muir (407) and by Caesar *et al.* (408). This system is strictly cellular in nature, with the few remaining myofibrils running continuously through the tissue, interrupted at the intercalated discs. Myosin has been detected immunologically in the fibrils [Helander & Emmart (409)].

#### CLOSING REMARKS

Of course, a reviewer likes to think that the topics he has just covered are of unusual interest. Even when discounting for this weakness, we believe that the past few years have brought essential progress in muscle physiology. It is not that any of the great problems have been brought to a final answer; on the contrary, we have been anxious to stress the fleeting nature of most of our current results and insights. But, above all, there has been a very promising change in emphasis. Work on the molecular constituents such as myosin and actin continues, but there is much more awareness of the necessity to connect this with the biological problem—mostly on the morphological level, for the time being. Also, work on the mechanical activity continues as before, but is now linked with biochemical and thermodynamic approaches. And finally, there are the first attempts to penetrate into the profound problem of the link between excitation and contraction. This change in approach, as Professor Weber has signalled in his preceding biochemical review (410, p. 669), rather than the individual results we have reported, constitutes great progress indeed.

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## KIDNEY, WATER AND ELECTROLYTE METABOLISM<sup>1,2</sup>

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### RENAL CIRCULATION AND GLOMERULAR FUNCTION

*The autoregulation of renal blood flow.*—The increase of intrarenal vascular resistance at increasing arterial pressures in the range of about 80 to 200 mm.Hg continues to receive widespread attention. In his excellent and concise review on present concepts of the renal circulation, Winton (183) holds that neither the cell separation theory of Pappenheimer & Kinter (113) nor the vasoconstrictor theory is apt to explain all the known experimental observations on renal hemodynamic autoregulation. For lack of alternative theories both must at present be thought to play their parts, yet some doubt has been shed recently on the existence and physiological significance of the phenomenon of autoregulation. In contrast to Weiss, Passow & Rothstein (178), who were able to demonstrate autoregulation of blood flow in the isolated rat kidney perfused with cell-free dextran solution, Ohler, Harth & Kreienberg (110) invariably found a straight-line relationship between arterial pressure and renal blood flow. Their rat kidneys were perfused with homologous or heterologous blood or a red cell suspension. Langston, Guyton & Gillespie (81) also failed to observe any sign of autoregulation of renal resistance in their series of kidneys perfused *in situ*. The authors tend to attribute this finding to their superiority of technique, since by cannulating the aorta from the caudal side they were able to avoid even a short interruption of renal blood flow. On the other hand, since the arterial pressures were maintained for periods of no more than two minutes at any one level, they might have missed the autoregulation which, as Grupp *et al.* (55) and others have shown, takes some time after the new pressure is instituted.

Extravascular pressure as a causal factor in the autoregulation of blood flow has been invoked by Hinshaw, Day & Carlson (63). Intrarenal pressures as measured with the needle technique disclose a near-linear relationship between the rate of rise of over-all renal resistance and tissue pressure throughout the autoregulation range. There also seems to be a correlation between the weight of the kidney and the vascular resistance.

Dextran-perfused kidneys show a similar degree of autoregulation correlated with increase in tissue pressure, indicating that the absence of red cells does not influence the phenomenon [Hinshaw *et al.* (61)]. From these and similar experiments performed on isolated perfused dog kidneys, Hinshaw &

<sup>1</sup> The survey of literature pertaining to this review was concluded in June 1960.

<sup>2</sup> Among the abbreviations used in this chapter are: ADH (antidiuretic hormone); NMN (N<sup>1</sup>-methylnicotinamide); and PAH (*p*-aminohippuric acid).



Carlson (62) conclude that the increased extravascular pressure within and without Bowman's capsule and, to a lesser degree, changes in blood viscosity resulting from glomerular filtration would suffice to explain the phenomenon of autoregulation although other factors might perform additional roles. Similarly, Scher (138) claims that autoregulation might be caused by the compression of some low-pressure vessels by extravascular fluid, since in his experiments on perfused dog kidneys the flow always paralleled the renal volume.

Grupp, Heimpel & Hierholzer (55) adhere to the vasoconstrictor theory without discussing other possibilities extensively. They specially draw attention to the passive change of renal blood flow immediately after a sudden increase or reduction of renal arterial pressure. Within a minute or so the renal resistance adapts itself to the new situation. The rise of arterial pressure following the release of carotid occlusion usually causes a slight increase of renal resistance to blood flow, yet autoregulation is still preserved [Grupp *et al.* (56)]. At the same time the renal heat production is diminished by an average of 12 per cent. Estimation of true values for intrarenal hematocrit is hampered by the tendency of plasma proteins to escape from the intravascular compartment, to a degree as yet undetermined. Emery and co-workers (41) have reasons to believe that this effect may be negligible. They analysed slices taken from various depths of dog kidneys, excised ten minutes after intravenous injection of  $^{51}\text{Cr}$ -tagged red cells suspended in plasma containing a fixed amount of  $^{131}\text{I}$  bound to human albumin. In the cortex the hematocrit averaged 47 per cent of systemic hematocrit with lower values in the outer than in the inner cortex. In the medulla the hematocrit was definitely lower (30%) whereas in the papilla it rose again to 47 per cent. The results are not much different from those of Lilienfeld, Rose & Lassen (92) except for the papilla in which the latter authors reported a hematocrit of only 8 to 9 per cent of arterial hematocrit. The presence of a low hematocrit in the medulla, but not the somewhat lower hematocrit in the outer than in the inner cortex, is consistent with the cell separation theory of Pappenheimer & Kinter (113).

It is important to note that the renal medulla apparently does not take part in the process of autoregulation. This has escaped attention so far since all experimental investigations have considered total renal blood flow and tacitly assumed that the hemodynamics of the medulla did not differ appreciably from the rest.

Kramer, Thureau & Deetjen (77) measured renal medullary and cortical circulation times electrophotometrically with selenium elements placed in the renal pelvis and below the capsule. After the injection of Evans blue in the renal artery, the mean circulation time averaged 2.5 seconds in the cortex and 27.7 seconds in the medulla. The length of time required for the passage of the dye through the medullary vessels is surprising at first, but easily understood if one considers that in the medulla of the dog kidney the capillaries cover distances of up to 20 mm. The increase of viscosity because of an increased protein concentration (82) may prolong the medullary circula-

tion time. It is noteworthy that, by means of a stalked Geiger-Müller counter, Kramer (76) found similar circulation times for the passage of  $^{32}\text{P}$ -tagged red cells through the medulla. This would contradict the assumption of a red-cell shunting mechanism for the medullary circulation.

From the circulation time the renal medullary blood flow can be computed. It is estimated that little more than 1 per cent of the total renal blood flow passes through the medulla. This is slightly less than the amount of fluid which is estimated to pass through the loops of Henle. Lilienfeld, Bauer & Maganzini (91) drew practically the same conclusion from their studies with  $^{131}\text{I}$ -labelled albumin. At increased renal arterial blood pressures, the circulation time is shortened in the medulla whereas in the cortex it remains constant [Thurau, Deetjen & Kramer (162)]. The autoregulation of renal blood flow apparently does not include the medullary blood flow. A 100 per cent increase of medullary blood flow caused by elevated renal arterial pressure would augment the total renal blood flow by little more than 1 per cent and pass undetected. Yet it might significantly influence the urine-concentrating mechanism. Increased flow along the vasa recta might affect the efficiency of the vascular countercurrent exchange system, and consequently reduce the degree of hypertonicity in the papilla. This might provide a very satisfactory explanation of the well-known phenomenon of pressure diuresis.

Similarly the passage time of dye is diminished, hence medullary blood flow increased, during both water diuresis and osmotic (glucose) diuresis. Since in both instances the medullary interstitial osmotic pressure fails to increase, the augmentation of blood flow may be fully explained by the absence of a transmembrane water shunt in the vasa recta. Whether an additional vasomotor effect plays a role remains to be established. Longley, Banfield & Brinley (93) draw attention to the close resemblance of the bundles of vasa recta in the outer zone of the mammalian renal medulla to the rete mirabile of the fish swim-bladder. The endothelia of the afferent (arterial) vessels show intense esterase activity whereas those of the efferent (venous) limbs do not. The authors hint at a possibility that the vascular bundles, being more than simple countercurrent exchangers, might contribute not only to the maintenance but to the creation of the hyperosmolarity of the renal papilla.

Green & Kepchar (53) in their review article on the control of peripheral resistance devoted a chapter to the renal vascular bed, with the conclusion that the kidney appears to contain only  $\alpha$  receptors, which are innervated by the sympathetic system and are highly sensitive to both epinephrine and norepinephrine. No evidence has been found for the existence of  $\beta$  adrenergic dilator or  $\gamma$  cholinergic dilator receptors for either hormonal or neural excitation.

Using arterial injection of India ink, Pérez-Tamayo & Hernández-Peón (114) took up the question of the patency of the glomeruli. Although their results confirm that in the undisturbed animals all glomeruli are patent, there are indications of an intermittent blood flow at an intraglomerular

level. With epinephrine and sympathetic stimulation, signs of vasoconstriction of the efferent and afferent arterioles and the interlobular arteries were produced.

By measuring the concentration of Evans blue in renal venous blood collected in samples of one second after quick injection of the dye into the renal artery, Mehrizi & Hamilton (99) calculated the renal vascular volume as the product of mean transit time and flow. During the infusion of norepinephrine, the well-known increase in kidney size is accompanied by an increase in blood capacity of the organ. This implies an increase in resistance in a segment of the renal vasculature which is downstream from the distensible part.

Emanuel and associates (40) reinvestigated the effect of 5-hydroxytryptamine on renal vascular resistance upon direct injection into the renal artery of the anesthetized dog. The observed increase in resistance was not dependent on extrinsic nerves and circulating or locally released catecholamines. Scott, Emanuel & Haddy (148) noted a decrease of total renal vascular resistance and increase in urine flow during the infusion of small amounts of potassium chloride into the renal artery of anesthetized dogs, causing the potassium content of renal venous plasma to rise to 10 m.eq. per liter. Larger amounts of potassium infused into the renal artery induced a progressive increase of renal resistance.

Pabst & Thron (112) reinvestigated the mechanism of the diuresis which is produced regularly in dogs during exposure to a cold (0 to 7°C.) environment. Since not only the urine volume is increased but also the output of sodium, chloride, and potassium, and since there exists a positive correlation between these values and the PAH and creatinine clearances, the changes are probably of primarily hemodynamic origin.

In the isolated kidney perfused at constant pressure from the original normothermic animal, a decrease of temperature resulted in an appreciable decrease of blood flow. This reduction of flow is not fully accounted for by the increased blood viscosity. The oxygen arteriovenous difference and metabolic oxygen consumption are also diminished [Levy (89)].

Katz (70) draws the attention to the much neglected yet important renal lymphatics. He was led to his investigation by the chance observation that the capsular lymphatics of dogs with pyelonephritis were markedly enlarged. A rise in lymphatic pressure in response to ureteral or renal venous obstruction was observed, suggesting that the renal lymphatics act as a safety valve when ureteral or venous pressure is raised. Le Brie & Mayerson (84, 85) undertook some studies on flow and composition of renal lymph. The protein content is 50 to 60 per cent of that of plasma, and electrophoretic analysis showed no significant difference between lymph and plasma in the distribution of albumin and  $\alpha_1$ ,  $\alpha_2$ ,  $\beta$ , and  $\gamma$  globulins. Sodium and chloride concentrations are consistently somewhat higher in lymph than in plasma. The elevation of abdominal venous pressure results in a significant rise of lymph flows and decrease in urine volume and urinary sodium concentration,

as was previously observed by Katz & Cockett (71). It is estimated that during elevated venous pressure as much as 50 ml. of lymph might be produced per hour by a kidney of a dog. These results emphasize that although renal lymph flow may be of no importance in normal conditions, it may become so under abnormal conditions of elevated venous pressure.

*Glomerular function.*—Vogel (172) compiled extensive data on the filtration rate and its relation to certain morphologic features such as number, diameter, and filtering surface of the glomeruli in five domestic animal species.

Schirmeister, Schmidt & Söling (139, 140), in an attempt to collect new evidence in favor of the reliability of inulin and creatinine clearances for the estimation of glomerular filtration rate, which in Germany is still questioned by a few (46, 60), studied the renal extraction ratio of inulin, creatinine, and PAH in anesthetized dogs during ureteral obstruction. Inulin and creatinine behaved in closely parallel fashion as expected. Both continue to be extracted (concentrations in renal venous blood smaller than in arterial) for as long as the ureter pressure rises. Under the conditions of mild osmotic diuresis prevailing in these experiments, the endpoint was reached after an average of 20 minutes at ureter pressures some 30 mm. Hg below (femoral) arterial pressure. The extraction, however, starts diminishing shortly after the ureter clamp is closed. The extraction of PAH, on the other hand, though slightly diminishing from the beginning, does not decline to zero for as long as 120 minutes. Renal blood flow behaves erratically, diminishing in some and rising in other instances.

That some filtration does indeed go on for several minutes after clamping of the ureter, even at the high rates of osmotic diuresis usually produced in stop-flow experiments, was shown by Omachi & Macey (111) by injecting two or more glomerular indicators (inulin, ferrocyanide, creatinine) at different times during the stop-flow period. If the test substance is injected early during the stop-flow period, it appears earlier (i.e., at a more distal site) after the release of the stop than if it is injected late during the stop period. Since in these experiments the ureter pressure rises to its maximum within 1 to 1.5 minutes, it must be concluded that some filtration (and reabsorption of water) goes on even at constant ureteral pressure.

Bálint & Forgács (3) give evidence that, at low filtration rates and extreme oliguria, the inulin clearance is not reliable since some of the filtered inulin accumulates in the kidney and is washed out when filtration rate and urine flow are raised.

#### URINE CONCENTRATION AND ANTIDIURETIC HORMONE (ADH)

*The countercurrent concentrating mechanism.*—The countercurrent theory of urine concentration has gained further support and now seems to be generally accepted. The most important evidence in its favor was contributed by Gottschalk & Mylle (52): in the golden hamster the contents of the loops of Henle near their hairpin bend show the same freezing point depression as

the contents of the collecting ducts at the same medullary level. A very favorable, well documented, and at the same time humorous review of the subject was written by Smith (154).

The theory is based in principle on the mathematical treatment and working model experiments of Hargitay & Kuhn (58), demonstrating that a small osmotic pressure difference continuously established between the contents of the limbs of a loop could be multiplied by countercurrent. In their working model, this primary osmotic pressure difference (*Einzeleffekt*) was created by a hydrostatic pressure head forcing water through a semipermeable membrane. From the mathematical elaboration it followed, however, that hydrostatic pressures which might be available in the kidney would not account for both the amount and the hypertonicity of mammalian urine during antidiuresis. Hargitay & Kuhn took into consideration an active water transport effected by the epithelial cells of the loops, possibly one of electro-osmosis. Today, the notion of active water transport being less popular than in the early fifties, the view prevails that the *Einzeleffekt* consists of an active sodium transport out of the ascending limb (52, 185), though direct evidence is still lacking. This modification of the original theory was presumed in a footnote to Hargitay & Kuhn's paper, which suggested that the same multiplication by countercurrent might ensue if the *Einzeleffekt* consisted of a transport not of water but of salt (of course in the opposite direction, i.e., from the ascending to the descending limb).

Recently Kuhn & Ramel (78) gave a mathematical description of a countercurrent multiplier system based on active salt transport. The equations arising from this treatment are highly complicated. It is clear, however, even to the mathematically ignorant that the concentrating factor (osmolar concentration ratio of urine to plasma) is an exponential function of the length of the loop and the solute transport activity, and inversely related to the volume of fluid entering the system per unit of time. Furthermore, there is a limiting interconnection between the concentrating factor, the transport activity, and the rate of isotonic fluid delivered to the collecting ducts. These conclusions are the same in principle as those derived from the mathematical treatment of the water-transporting countercurrent system by Hargitay & Kuhn (58).

The well-known diminution of urine osmotic pressure in osmotic diuresis may have one or both of two causes: (a) inadequacy of proximal reabsorption, resulting in an increased delivery of isotonic fluid to the countercurrent system of the loops; (b) inadequacy of distal reabsorption, causing an increased rate of flow to the collecting ducts.

In the dog the amount of isotonic fluid delivered to the countercurrent system of the loops under normal conditions of hydropenia or antidiuretic hormone loading is still too large to permit the production of maximally concentrated urines. By moderately reducing renal arterial pressure and therefore glomerular filtration rate, Levinsky, Davidson & Berliner (87) obtained a further increase of urine osmolar concentrations even when control urine

concentration was as high as 2000 mOsm. per kg. H<sub>2</sub>O. If, however, the reduction of filtration rate was 30 per cent or more, urine osmolality fell markedly, but was readily restored by inducing a mild mannitol or urea diuresis. This phenomenon is not predicted by the equations of Kuhn & Ramel. It may be understood by following the reasoning of Levinsky *et al.* that the fraction of filtered sodium reabsorbed in the proximal tubule presumably increases when the filtration rate is reduced, and less is available for the transport by the loops of Henle. Hence the medullary interstitial sodium concentration falls. An alternative or additional reason for the reduced urine osmolalities might be that too little sodium remains available for reabsorption in the distal convoluted tubules; hence the distal volume restriction is hampered and too much solute is delivered to the collecting ducts relative to the flow through the loops.

It is clear that no multiplication of the *Einzeleffekt* can occur if the flow through the loops is abolished altogether as in stop-flow experiments. Samples of urine coming from the far distal areas (possibly the collecting ducts) showed a small but significant rise of osmotic pressure of about 10 per cent of the total urine osmotic pressure. Kessler and associates (75) were disappointed not to find any indication of the expected distal tubular concentrating activity and suggest that, for some unknown reason, the stopflow method fails when one uses it in an attempt to study the renal concentrating mechanism. However, Malvin & Wilde (95) in reinvestigating this phenomenon interpreted it as strongly in favor of the countercurrent hypothesis: if there existed a "water pump" in some distal area, responsible for urine concentration, one would expect the urine lying in this area to become more and more concentrated during the stop-flow period. Since this does not occur, the result suggests strongly that this type of concentrating mechanism plays no role in renal function.

It has long been suspected, as predicted from the equations of Kuhn *et al.*, that there exists a positive correlation between the length of the loops (for the incidence of long loops in the nephron population) and the urine-concentrating capacity of different mammalian species, although most earlier publications were done on a speculative basis. Schmidt-Nielsen (109) re-investigated the subject using three characteristic mammalian species under the stress of severe dehydration. Beavers with short looped nephrons showed maximum urine osmolalities of only 600 mOsm., rabbits with both long and short loops performed about as well as man (1500 mOsm.), and *Psammomys obesus* with only long loops reached the ceiling at 6000 mOsm. Apparently both long and short loops act as countercurrent multipliers. That the inner zone is also a multiplier system is indicated by the continuous increase in sodium concentration through this zone and the close relationship between the thickness of this zone and the concentrating ability. Similarly, the sodium content of the medulla varies with the number of long loops [Barclay, Cramp-ton & Matthews (4)]. The cat and dog with 100 per cent long loops show the highest sodium content, the pig with practically no long loops is lowest, and

the rabbit and rat with a mixture of long and short loops have intermediate sodium contents.

Morel, Amiel & Falbriard (101) approached the problem by combining the stop-flow technique in the anesthetized rabbit with an analysis of the kidneys *post mortem*. The most important of their findings is that during the "stop" period, tritiated water does not reach the deep renal medulla within three minutes whereas  $^{24}\text{Na}$  does. This seems to indicate that the hairpin system of the vasa recta provides a nearly perfect countercurrent exchange system as far as water goes, but is definitely less effective as an exchanger of sodium. During the polyuria which follows the stop-flow period, the concentration of tritiated water in the urine is at all times similar to that existing in the deep medulla and papilla. This indicates that the walls of the collecting ducts have a very high permeability to water molecules.

In similar experiments injecting  $^{24}\text{Na}$  or  $^{40}\text{K}$  in the middle of a stop-flow period of six minutes duration, Morel & Falbriard (102) were able to demonstrate that the collecting ducts and the distal tubules, including an area of considerable active sodium reabsorption, are impermeable to sodium in the direction from interstitial space to lumen, whereas the adjacent more proximal tubules are extremely permeable to this ion. In the case of potassium, the collecting ducts seem to be impermeable as well. An area of high potassium permeability is located more distally than that of sodium permeability and coincides with the region of enhanced sodium reabsorption.

In the dehydrated hamster, Morel, Guinnebault & Amiel (103) reached the same conclusion by estimating  $^{22}\text{Na}$ , HTO, and total sodium in 10 to 11 different zones from cortex to papilla at different times after injection of the indicators. One minute after the injection, the specific activity of sodium of the medulla is identical with that of the cortex except for the papilla where equilibrium is attained in two minutes. The replacement of medullary water proceeds at a much slower pace; in fact, ten minutes after the injection the concentration of HTO in the deep medullary regions is still inferior to that in the cortex. Thus water is short circuited much more efficiently than sodium by the countercurrent exchange system of the medullary vessels. In osmotic diuresis, on the other hand, HTO is nearly evenly distributed throughout the medulla as early as two minutes after the injection. This again indicates that water is delivered to the medullary tissue by way of the collecting ducts rather than the vasa recta.

There remain a number of difficulties still to be solved. One of them is that a good deal of the osmotic work should be placed in the flat epithelial cells of the thin limbs of long loops; and another, even weightier one is that the thin limb should change its functional capacity at the tip of the loop "for no better reason, apparently, than the circumstance that it has turned a corner" [Smith (154)].

Ullrich (165) thought he had solved both these problems by assigning the active part of the countercurrent concentrating mechanism exclusively



to the thick ascending limb of the loop and consequently to the outer medullary zone. He was led to this conclusion by the findings of Ullrich & Pehling (170) that the oxygen consumption of slices of the outer, but not of the inner, medullary zone increases with increasing sodium concentrations of the medium. He is faced, however, with new difficulties. The terminal step of urine concentration he describes as attributable to the reabsorption from the collecting ducts of a fluid which is hypo-osmotic as compared with collecting duct and medullary interstitial fluid (but not necessarily to systemic blood). This would imply the reintroduction into renal physiology of the notion of active water transport. Alternatively, he speculates (164) that the active concentrating process might be confined to the outer medullary zone, the inner zone serving mainly for the completion of passive equilibration processes. This seems to be contradicted by a number of experimental results of his own [Ullrich, Drenckhahn & Jarausch (166)] and others (86, 103, 187).

A clinical problem which might have a connection with the countercurrent concentrating mechanism concerns the impairment of concentration capacity in patients with sickle cell anemia. In such children, Whitten & Younes (181) found essentially normal values for solute-free water reabsorption ( $T_m^c H_2O$ ) during osmotic diuresis, whereas  $TcH_2O$  and concentrating capacity in the hydropenic state were defective, as noted by a number of earlier observers. The phenomenon is discussed in the light of the modern concept of urine concentration, and most possible explanations are dismissed as unlikely except for the possibility of anoxic damage of some medullary epithelial cells secondary to the vascular occlusive phenomenon associated with intravascular sickling.

*Influence of calcium and potassium on the concentrating mechanism.*—The influence of acute hypercalcemia on kidney function has been described as causing reduced reabsorption of sodium [Levitt *et al.* (88); Wolf & Ball (188)] and polyvalent cations (188). The polyuria and decrease in urine osmotic pressure which are most commonly observed in the state of hypercalcemia are thus, at least partly, the consequence of osmotic diuresis. In addition, there seems to result an impairment of water reabsorption from the collecting ducts since Beck, Levitin & Epstein (8) demonstrated a decrease of solute-free water reabsorption ( $TcH_2O$ ) resulting from intravenous infusion of calcium gluconate in normal dogs undergoing mannitol diuresis.

More severe defects of the concentrating capacity were found by Epstein *et al.* (42) in dogs in which hypercalcemia had been induced for 24 hours by injecting parathyroid extracts. During moderate mannitol diuresis, these dogs excreted a hypotonic urine despite the infusion of exogenous vasopressin. According to Epstein *et al.* the impairment of concentrating ability might result from a decrease in sodium reabsorption from the ascending limb with consequent disruption of the countercurrent concentrating mechanism or the slowing of the back diffusion of water from the collecting ducts to the hypertonic interstitial fluid of the medulla, or both.

Carone and co-workers (23) presented a more detailed description of the morphological alterations which were confined to the thick ascending limb of the loop of Henle, the entire distal convolution, and the collecting ducts. When serum calcium fell to normal after 48 to 72 hours, the reabsorption of solute-free water and concentrating capacity returned to normal in some animals, but blood urea nitrogen remained elevated and even rose further, and the morphologic lesion persisted. In other animals the reabsorption of solute-free water remained low after serum calcium level had returned to normal. It is postulated that the acute functional changes are ascribable to the inhibitory effect of excess calcium upon cellular permeability, preventing the passive reabsorption of water from making the urine hypertonic. The morphologic alterations may arise from an effect of excess calcium on the cell metabolism. Functional and morphologic changes are not necessarily correlated. Similar functional observations have been made in man by Lambie & Robson (80), who suggest that this limitation of water conservation may be partly responsible for the polyuria commonly found in patients with hyperparathyroidism.

By comparing the composition of renal cortex, medulla, and papilla with that of plasma and urine, Manitius *et al.* (98) studied the mechanism of this concentrating defect in hydropenic rats, in which hypercalcemia had been induced by large doses of vitamin D. Since the content of sodium and urea in the papilla and medulla of these rats was significantly decreased, it is concluded that the hyposthenuria results at least in part from the impaired reabsorption of sodium and a diminished ability to create and maintain high sodium concentration in the medullary interstitium. The tendency of vitamin D-treated rats to lose sodium on a sodium-free diet further suggests that actual reabsorption of sodium by some part of the nephron is impaired. The possibility is not excluded, however, that the obstruction of the back diffusion of water from the collecting ducts may also play its part. The finding of hypotonic urines during mannitol diuresis in hypercalcemic dogs (42) strongly suggests this assumption.

Sanderson (132) reported loss of maximum urinary concentrating capacity in rats on acid high-calcium, high-phosphorus diets. These animals showed no rise in serum calcium or phosphorus but a marked increase in the calcium content of the kidneys with or without histological evidence of calcification in the region of the collecting ducts.

In the stop-flow experiments of Howard, Wilde & Malvin (65), no apparent influence on the distal sodium reabsorption was noted at high plasma calcium levels. However, the striking diminution of water reabsorption—since in stop-flow experiments no water reabsorption by the countercurrent mechanism occurs—suggests some reduction of the reabsorption of sodium.

A marked decrease in the ability of the kidneys to excrete highly concentrated urine similar to that found in hypercalcemia is known to occur regularly in hypokalemic states [Hollander *et al.* (64); Relman & Schwartz

(125)]. This decrease was also found in rats by Senft & Natzschka (150) when a severe potassium deficit was induced by repeated sodium infusions. The mechanism of this hyposthenuria was studied in dogs and rats by Manitius and associates (97), using the same method reported in detail (98) for hypercalcemic rats. Since the ratio of urinary solute concentration to papillary solute concentration was uniformly lower in potassium-deficient animals than in normal animals, it is concluded that potassium deficiency impairs the permeability of the collecting ducts to water. It cannot be stated, however, that this is the only reason for the hyposthenuria.

The reduced sodium excretion in hypokalemic dogs following prolonged steroid treatment was attributed by Poutsika, Nasveschuk & Millstein (121) to a marked reduction in glomerular filtration rate.

*Site and mechanism of action of antidiuretic hormone (ADH) in the mammalian kidney.*—These problems still bear a number of question marks. It is generally recognized that at least part of the action of ADH is permissive, facilitating the passive transfer of water across some renal structures. Among these the collecting ducts and the distal convoluted tubules are well established as target structures (52, 87, 185). But the action of ADH on the descending limb of the loop is still a matter of debate, and the exact part played by this segment of the nephron in the countercurrent mechanism is not definitely ascertained.

Autoradiograms of isolated nephrons from rats injected with  $^{125}\text{I}$ -labelled pitressin show that the radioactivity is located over the distal convoluted tubule and the collecting duct [Darmady *et al.* (30)]. These are the locations where, according to the present knowledge, ADH exerts its activity by increasing the permeability to water. Proximal convoluted tubules and the thick ascending limb of the loop were devoid of activity. Unfortunately, the thin segments of the loops of Henle including the descending limb were too delicate to permit the preparation of autoradiograms.

Ginetzinsky (48) proposed that ADH acts on some renal structure by activating hyaluronidase, which in turn facilitates the passive transfer of water. Berlyne (18) questions the results of Ginetzinsky (48) and his group on technical grounds, saying that precise estimation of hyaluronidase by any viscosimetric method should be done at constant electrolyte composition and that Ginetzinsky's method of assigning arbitrary units of hyaluronidase activity is open to error. In his own experiments during antidiuresis, water diuresis, mannitol or potassium diuresis, he found no correlation between hyaluronidase excretion (expressed in units per minute) and the rate of urine flow. Leaf (83) found that hyaluronidase, if applied to the isolated toad bladder, had no influence on the permeability of this membrane to water and urea, or on the active sodium transport.

Sawyer, Munsick & van Dyke (135) presented evidence that the vasopressor-water balance principle of birds, reptiles, amphibians, and teleosts is identical with "arginine vasotocin". This compound, synthesized by Kat-

soyannis & du Vigneaud (68, 69), is an octapeptide containing the ring structure characteristic for oxytocin, attached to a side chain of arginin vasopressin. The compound seems to be identical with the eluate B from fish and frog pituitaries prepared by Pickering & Heller (118), showing both oxytocic activity in mammals and water balance activity in the frog. "Arginine vasotocin" is many times more active than either vasopressin or oxytocin in accelerating the passage of water across the urinary bladder of the bullfrog [Sawyer (134)]. "Oxypressin" on the other hand, a synthetic compound containing phenylalanine in position 3 of the ring structure as vasopressin, but the side-chain characteristic for oxytocin, has been found by Berde, Daepfner, and Konzett (9) to have an antidiuretic activity in the rat comparable to that of vasopressin. A similar antidiuretic potency was found in man by Thomson (159). According to Thorn (161), the antidiuretic action of "oxypressin" is somewhat shorter in the rat and the dog, whereas lysine vasopressin, the vasopressin variety of the hog, is both less potent and shorter acting.

Since neurohypophyseal extracts of all vertebrates contain oxytocic, vasopressor, and antidiuretic activities, it was tempting to look for homologous structures in invertebrates. Sawyer (133) demonstrated weak oxytocic activities in extracts from the neural complexes of two ascidian species. However, the resemblances of the activities of these extracts to those of the vertebrate neurohypophysis are too superficial to support the argument for homology of these organs.

Schröder & Rott (144) once more took up the question of specificity of biological assay methods for ADH activity. In plasma of normal and edematous persons, the ADH activity never reached the level of 10 to 20  $\mu$ U per ml., the limit for specific assay methods. Higher values which continue to be reported in the literature are caused by nonspecific antidiuretic action and not by the hormone. Under this aspect even the results of Buchborn (22), showing a correlation of water retention, plasma osmotic pressure, and ADH activity of plasma must be questioned. The same conclusion as to potency and specificity of biologic assay methods and the absence of true ADH activity in human plasma was drawn by Vorherr & Friedberg (175).

Normal human plasma does not reduce the antidiuretic activity of vasopressin added *in vitro*. Plasma of pregnant women does, however, the highest inactivating activity being found during the last third of pregnancy [Vorherr & Friedberg (176)]. Thorn (160) collected evidence on biochemical and physiological grounds that the antidiuretic substance recovered from rat urine after loading with hypertonic salt solutions is indeed vasopressin. It was not decided, however, whether this compound is of the arginine or the lysine type. Stelter (157) reports that in the mouse, as in the rat, vasopressin exhibits its usual antidiuretic effect if the diuresis is produced by water but not by 0.9 to 1.8 per cent salt solutions. Unlike the rat, however, the mouse produces neither natriuresis nor diuresis after the administration of oxytocin.

## TUBULAR FUNCTIONS

*Excretion of water and strong electrolytes.*—The old controversy as to the significance of plasma colloid osmotic pressure in the reabsorption of water had received new impetus by the suggestions of Bayliss (184), Malvin *et al.* (96), and others that the osmotic pressure of the plasma proteins of the peritubular capillaries could provide a force sufficient for the bulk of water reabsorption from the proximal tubule. While it is certainly true that mathematically the oncotic pressure is large enough to account for the tubular water reabsorption, provided the permeability of the tubular wall be large enough, evidence seems to accumulate that an overwhelming fraction of water is reabsorbed secondarily to an active solute reabsorption.

The method of stopped flow microperfusion introduced by Shipp and co-workers (151) in 1958 provided a very valuable tool. With its use Schatzmann *et al.* (137) were able to demonstrate that the reabsorption of water from single proximal tubules of *Necturus* was inhibited by 2,4-dinitrophenol and ouabain. Apparently a cellular metabolic activity is involved in the proximal reabsorption of water, and the results are in agreement with the assumption that water reabsorption is secondary to an active transport of sodium.

With the same preparation, Windhager and associates (182) were led to conclude that movement of water from the tubule is passive and secondary to active solute transport, since net water flux may be accounted for quantitatively in terms of osmotically induced forces. Net water flux was zero when net solute flux was zero. The volume and direction of movement of water showed an approximately linear relationship to the sodium chloride concentration in the lumen, if the perfusion fluid was made isotonic with mannitol. Thus, in these experiments water movement was secondary to salt movement and therefore a passive process.

Whittembury *et al.* (180) took advantage of the same method to study the relationship between net water flux and osmotic gradients across the proximal tubule of *Necturus*. From this the permeability coefficient for water was estimated to be  $0.15 \times 10^{-8}$  ml./( $\text{cm}^2 \times \text{sec.} \times \text{cm. H}_2\text{O}$ ). The tubule wall is thus relatively tight, the permeability coefficient being smaller by two orders of magnitude than that of the frog glomerulus. The plasma colloid osmotic pressure of *Necturus* amounts to some 9 cm.  $\text{H}_2\text{O}$  which would account for less than 2 per cent of the average net water reabsorption observed when the tubule is filled with an isomolar salt solution. The conclusion that the osmotic pressure exercised by the plasma proteins is too small to play a significant part in water reabsorption has been confirmed in experiments in which albumen was added to the fluid inside the tubule. In these experiments the direction of protein osmotic pressure gradient was reversed, yet the absorption of water continued in the usual direction.

Giebisch (47) by carefully compiling the available evidence (his own and that of others) describes the transport situation of the proximal convoluted tubule of *Necturus*. Measurements of electrical potential differences across

single tubules indicate that the tubule lumen is electrically negative to the outside by some 20 mv., and that the interior of the cell is negative with respect to both the peritubular and the tubular fluid. It is suggested that the electrical negativity of the interior of the tubule cell is created by the formation, within the cell, of an area rich in potassium and low in sodium. The transmembrane potential difference at the contraluminal cell membrane is close to that to be expected from the concentration gradient of potassium across this membrane. It behaves like a potassium diffusion potential and is diminished if the potassium concentration in the peritubular fluid is raised. In fact, above a concentration of 5 m.eq. per liter there is a straight-line relationship between transmembrane potential difference and the logarithm of external potassium concentration. This is indicative of considerable permeability of the membrane to potassium.

An active sodium transport system is located at the contraluminal, but not—according to Giebisch—at the luminal cell membrane. Both the luminal and the contraluminal membranes are permeable to some extent to sodium, the latter relatively less than the former. It is further suggested that the passive diffusion of sodium at a higher rate than chloride and an active uptake of potassium from the tubule lumen to the cell partially shunt the potential difference across the cell and at the same time render the inside of the tubule electrically negative. Since the tip of the Ling-Gerard electrode cannot be seen at the magnifications ordinarily used for electrophysiological measurements of single cells or nephrons, the position of this tip is usually ascertained by the injection of dye-stuffs. Eigler (39) describes a method which is based on the changes of the electrical resistance during the passage of a silicone oil droplet which is injected into Bowman's capsule.

Karger (67) modified the short-circuit method of Ussing & Zerahn (171) for the evaluation of the transport of electrically charged solutes. His device, using two instead of four electrodes, permits the application of this method on a micro scale, where the use of four single electrodes would be impossible.

Again it was ascertained that the urinary bladder of the dog is not a perfect barrier to electrolytes [Rapoport, Nicholson & Yendt (122)]. The movement of electrolytes into and out of the bladder appears to be passive. The net transfer of  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Cl}^-$ , and  $\text{H}^+$  depends on the direction and magnitude of their concentration gradients with plasma.

*Acid-base regulation.*—Valuable new information has been gained on the dynamics of bicarbonate reabsorption in the dog by Schwartz and co-workers (145 to 147). Acute reduction of plasma  $\text{pCO}_2$  to approximately 20 mm.Hg by hyperventilation (producing respiratory alkalosis) depresses the reabsorption of bicarbonate to an average of 1.9 m.eq. per 100 ml. of filtrate. As at normal plasma  $\text{pCO}_2$ , the reabsorption is essentially constant over a wide range of plasma bicarbonate concentrations [Schwartz, Lemieux & Falbriard (147)]. Elevation of plasma  $\text{pCO}_2$  to 90 to 100 mm.Hg (by producing respiratory acidosis) does not simply elevate the reabsorption capacity to a new

constant rate, but bicarbonate reabsorption becomes a curvilinear function of plasma bicarbonate level [Schwartz, Falbriard & Lemieux (145)]. The results are consistent with the assumption that bicarbonate reabsorption takes place by means of a two-step process involving a nonenzymatic and an enzymatic component, either of which may be rate limiting. A normal or diminished  $p\text{CO}_2$  limits the reabsorption to a fixed rate and prevents the full utilization of the enzyme. In respiratory acidosis this limit moves upward and the enzyme step becomes rate-limiting. Similarly, the enzyme step is rate-limiting if carbonic anhydrase is partially inhibited by acetazolamide [Schwartz, Falbriard & Relman (146)]. In accordance with this hypothesis (though not proving it), the relationship between plasma bicarbonate concentration and the rate of reabsorption in respiratory acidosis takes the same form as the Michaelis-Menten equation which describes the initial velocity of an enzymatic reaction as a function of substrate concentration.

Seldin *et al.* (149) draw attention to the fact that a similar reciprocal relationship between plasma bicarbonate concentration and bicarbonate reabsorption could arise from competition between bicarbonate and non-bicarbonate buffer systems for secreted hydrogen ions. By various alterations of the acid-base balance in normal subjects, they further found that after the administration of acetazolamide the bicarbonate reabsorption varied linearly with plasma  $p\text{CO}_2$ . Rector & Seldin (124), from studies on dogs at different plasma  $p\text{CO}_2$  with and without acetazolamide administration, draw the conclusion that carbonic anhydrase contributes a constant fixed amount to  $\text{HCO}_3^-$  reabsorption at all plasma  $\text{HCO}_3^-$  concentrations. Accelerated  $\text{HCO}_3^-$  reabsorption during respiratory acidosis is attributable solely to the uncatalyzed reaction, and all  $\text{HCO}_3^-$  reabsorption, regardless of plasma  $\text{HCO}_3^-$  concentration, is dependent on plasma  $p\text{CO}_2$ .

In contrast to the findings of Schwartz *et al.* (145), prolonged exposure to atmospheres containing 7 to 10 per cent  $\text{CO}_2$  elevates the reabsorption of bicarbonate to a rate which is constant at a wide range of plasma bicarbonate concentrations, whether the experiment is performed in an atmosphere of elevated or normal  $\text{CO}_2$  content [Toussaint, Telerman & Vereerstraeten (163)]. The authors tend to believe that the phenomenon is related to the hypochloremia found regularly in these dogs, since the reabsorption of bicarbonate rises to approximately the same degree if the dogs are made hypochloremic by peritoneal lavage.

Simmons, Assali & Avedon (152) found that, in the anesthetized dog, increase in plasma bicarbonate concentration (by induction of metabolic alkalosis) produces a rise in urinary pH and an increase in renal bicarbonate excretion even if a rise in arterial pH is prevented by hypoventilation (causing respiratory acidosis). Inversely, urine pH dropped and renal acid excretion increased in metabolic acidosis induced by hydrochloric acid infusion but compensated as to arterial pH by hyperventilation (respiratory alkalosis). In other words, the changes in plasma bicarbonate concentration are of



greater influence on the secretion of hydrogen ions and bicarbonate reabsorption than changes of arterial  $p\text{CO}_2$  comparable in terms of their effect on arterial pH.

Previous experiments of Simmons & Avedon (153) had shown that the plasma potassium level is not changed by similar acid-base alteration, provided the arterial pH is kept constant by adapting the respiration. If, however, the pH is altered, the plasma potassium changes inversely an average of 2 m.eq. per liter for each unit change in pH. Achieving a steady state requires one to two hours. It is concluded that the extracellular potassium concentration is physiologically regulated and that this involves exchanges with intracellular potassium.

In the anesthetized rat without diuresis, Gottschalk, Lassiter & Mylle (51) found by micropuncture experiments that a certain pH drop occurs as high as the proximal convoluted tubules. In the distal convolution the tubule fluid is usually slightly acidic, but the greatest fall occurred beyond the distal convolution, in the collecting ducts. This is in good agreement with the results obtained by microcatheterisation by Ullrich & Eigler (167) which disclosed a definite drop of pH in the collecting ducts. In osmotic diuresis induced by mannitol or glucose, the final drop of pH in the collecting ducts was much smaller. After ammonium chloride loading, the pH drop along the proximal convolution was very marked. This latter finding, according to Gottschalk *et al.*, does not necessarily signify an increased proximal  $\text{HCO}_3^-$  reabsorption, since at a low concentration of  $\text{HCO}_3^-$  in plasma and filtrate, as in the acidotic rat, equivalent bicarbonate reabsorption leads to a greater decrease in pH.

The largest part of bicarbonate reabsorption (probably caused by exchange of  $\text{Na}^+$  for  $\text{H}^+$ ) must occur in the proximal convoluted tubule, since approximately 80 per cent of the filtered water is reabsorbed proximally and the drop in pH indicates that relatively more bicarbonate is reabsorbed than water. On the other hand, although the greatest drop of pH occurs in the collecting ducts, this may be caused by a relatively low rate of  $\text{H}^+$  secretion, since the amount of fluid presented to the collecting ducts is small.

By stop-flow experiments in acidotic dogs, Sullivan, Wilde & Malvin (158) located the excretion of titratable acid in a far distal site of the nephron, probably in the collecting duct. At the same site the concentrations of potassium and ammonium ions are highest.

The intriguing and controversial fact that  $p\text{CO}_2$  in alkaline urine is higher than in venous blood (74, 119) has been reinvestigated in man by Portwood *et al.* (120). Because a difference of about 30 mm.Hg between urine and plasma partial pressures was found even during water diuresis, when buffer excretion was minimal, they conclude that the difference is caused by delayed dehydration of  $\text{H}_2\text{CO}_3$  to  $\text{CO}_2$  and  $\text{H}_2\text{O}$  as proposed by Pitts & Alexander (119). Kennedy, Eden & Berliner (73) had shown that the dehydration of  $\text{H}_2\text{CO}_3$  is immeasurably rapid in the absence of buffer *in vitro*. Indeed, the increase of buffer excretion after phosphate load raised the urine to plasma gradient, giving moderate support to the view that buffers can increase

the urinary  $\text{CO}_2$  tension by delaying the dehydration of  $\text{H}_2\text{CO}_3$ . For similar reasons Rector, Portwood & Seldin (123) proposed that the mixing theory of Kennedy, Orloff & Berliner (74) is a very improbable explanation of the elevated  $\text{CO}_2$  tensions.

In severe acute respiratory acidosis ( $\text{pCO}_2$ , 100 to 120 and 170 to 190 mm.Hg) Kaim & Brodsky (66) obtained inconsistent results, urine  $\text{pCO}_2$  being greater than, equal with, or smaller than plasma  $\text{pCO}_2$ . Injection of carbonic anhydrase failed to obliterate the gradient if urine  $\text{pCO}_2$  was smaller than plasma  $\text{pCO}_2$ . Inversely, in a set of experiments on alkalotic dogs, the results of Ochwaldt & Pitts (108) that urine  $\text{pCO}_2$  was diminished to the level of plasma  $\text{pCO}_2$  after the infusion of carbonic anhydrase were confirmed. This problem cannot be settled definitely before more is known concerning the  $\text{pCO}_2$  in blood and interstitial fluid of the renal papilla, since it is very likely that  $\text{CO}_2$  is trapped in the renal medulla by the countercurrent exchange mechanism of the vasa recta during alkalosis, or, inversely, that the medullary  $\text{pCO}_2$  decreases in the state of acidosis, when more  $\text{CO}_2$  is used for urine acidification than is produced locally.

Very much the same problem arises with regard to oxygen. Rennie, Reeves & Pappenheimer (126) have noted that urinary  $\text{pO}_2$  may be lower than venous  $\text{pO}_2$ . While this is consistent with the cell separation theory (113), it might, at least partly, be explained by an oxygen shunt at some renal capillaries, especially those of the vascular bundles of the outer medulla. Levy & Saucedo (90) noted that, after the injection of a mixture of methemoglobinemic cells in highly oxygenated blood into the renal artery, the appearance time of oxygen in renal venous blood was shorter relative to cells.

*Ammonia and glutaminase I.*—Ammonia secretion was shown to occur in the collecting ducts of the hamster by Ullrich, Hilger & Klümper (168). This was a confirmation of the conclusion of Richterich & Goldstein (128) from their high estimates of glutaminase I activity in dissected kidneys. However, the results of Weiss & Longley (177) indicate that there is not enough glutaminase I present in the inner medulla to account for the amounts of ammonia which can be excreted. Therefore they question whether glutaminase I is the immediate metabolic agent for release of ammonia in the rat. Goldstein & Copenhaver (50) find increased glutaminase I activity in rat renal homogenates when the ammonia excretion is raised by repeated ammonium chloride administration. It appears that the concentration of glutamic acid in the kidney regulates the enzyme synthesis, since the rise in glutaminase I activity as well as that of ammonia excretion after ammonium chloride is suppressed by the injection of sodium glutamate. In the alligator, the chief function of ammonia production by the kidney seems to be the urinary excretion of nitrogen whereas the conservation of sodium is incidental [Coulson & Hernandez (27)].

*Potassium.*—Using the stop-flow technique, Sullivan, Wilde & Malvin (158) showed that potassium secretion occurs at a very distal site, probably in the collecting ducts, since the very first samples collected after the release of

the ureteral obstruction were more concentrated than plasma. The point of maximum potassium concentration was more distal than that of minimal sodium concentration. From there the potassium concentration fell abruptly to show a minimum at, or just proximal of, the site of minimal sodium concentration. Later samples arising from proximal tubular sites consistently showed potassium concentrations above the plasma level. This seems to indicate that the site of active potassium reabsorption as postulated from indirect evidence by Berliner, Kennedy & Hilton (17) takes place at a site more distal than was concluded from the experiments of Bott (20) and Wirz & Bott (186), but more in keeping with the later observations of Bott (19) in *Necturus*. From studies of simultaneous clearances of rubidium and potassium, Kunin *et al.* (79) concluded that rubidium and potassium are probably handled by similar or identical mechanism.

Intravenous infusions of magnesium and calcium salts, regardless of the anions, cause marked increase of potassium excretion in dogs. The stop-flow experiments of Samiy, Brown & Globus (130) gave equivocal results as to the mechanism of this effect; they can be interpreted as indicating depression of potassium reabsorption in the proximal segment, increased secretion in both the proximal and the distal segments of the nephron, or both.

A number of purely herbivorous domestic animals show spontaneous potassium clearances which are approximately as high as the glomerular filtration rate. By the infusion of potassium chloride it is easy to raise the potassium clearance considerably above filtration rate [Vogel (173)].

*Excretion of calcium, magnesium, and phosphate; action of parathyroid hormone.*—No exciting news has been reported on the renal action of parathyroid hormone since the convincing conclusions of Nicholson (106) that parathyroid hormone acts on the distal convoluted tubule by stimulating the secretion of phosphates. A direct positive effect of the hormone on the tubular reabsorption of calcium is claimed for the mouse by Buchanan, Kraititz & Talmage (21). Carrasquer & Brodsky (24) presented evidence for tubular secretion of inorganic phosphate in the dog, using a method similar to that described by Chinard (25). Their way of presenting the data, however, does not allow any conclusion concerning the location of this secretion in the nephron.

By stop-flow experiments at elevated plasma calcium levels, Howard, Wilde & Malvin (65) demonstrated a distal site of calcium reabsorption close to but slightly proximal to the site of maximal sodium reabsorption. Wesson & Lauler (179) found this site to be identical with that of maximal magnesium reabsorption. The distal site of active calcium and magnesium reabsorption was also confirmed by Samiy and co-workers (131). The excretion of calcium is increased by the infusion of magnesium chloride, and the excretion of magnesium by calcium chloride infusions, although to a lesser extent. This supports the postulate that calcium and magnesium compete for a common reabsorptive system in the renal tubule. By injecting  $^{28}\text{Mg}$  two minutes before releasing the clamp in stop-flow experiments, Ginn *et al.*

(49) found that the radioactive magnesium appeared earlier than the filtered substances and earlier than the peak of *p*-aminohippuric acid concentration. They conclude that magnesium is secreted by the distal tubule. Experiments of that kind do not, however, permit any conclusion on the net magnesium flux; they simply permit a statement that the nephron is permeable to magnesium. This is the conclusion drawn by Murdaugh & Robinson (104) from similar experiments. By the ordinary stop-flow procedure, using  $^{25}\text{Mg}$ , they found  $^{25}\text{Mg}$  urine to plasma ratios below unity in the samples originating from early distal tubules indicating an active magnesium reabsorption at that site. It coincides with the site of maximal calcium reabsorption and is slightly proximal to the site of minimal sodium concentration in stop-flow samples [Wesson & Lauler (179)].

*Excretion of organic bases.*—Peters (117), reviewing the tubular excretion of organic bases, enumerates 14 strong bases including amines and quaternary ammonium compounds, half of them synthetic chemicals and half occurring naturally in the tissues and urine of vertebrates. The compounds most extensively used are  $\text{N}^1$ -methylnicotinamide (NMN) [Sperber (156)], tetraethylammonium, and mepiperphenidol (Darstine). The tubular extraction of some of these substances may be as high as that of *p*-aminohippuric acid. Only slight tubular excretion is found in the case of hexamethonium.

There is reason to believe that all of these strong bases might reach a maximal rate of transfer ( $T_m$ ), though the evidence is weak in some instances because of the strong pharmacodynamic activity or toxicity of some of the compounds. All these bases apparently share a single transport system which is different in at least some of its components from that which effects the transport of *p*-aminohippuric acid, phenol red, and iodopyracet (Diodrast).

Similarly, in the chicken, NMN and several homologues of tetramethylammonium are excreted by the same tubular transport system [Green *et al.* (54)]. Mepiperphenidol is a potent inhibitor for the transport of NMN. Among a number of 1-alkyl-1-methylpiperidinium compounds, the ability to inhibit the NMN transport increases as the length of the chain of one alkyl substituent is increased [Volle *et al.* (174)]. In the dog, after a prolonged period of forced-feeding, the tubular secretory capacity increases for some of these bases (tolazoline, NMN) but not for others (mepiperphenidol) [Domer (38)]. The excretion efficiency for mecamlamine is increased by forced-feeding of potassium.

*In vitro* studies on kidney slices with a technique similar to that used by Cross & Taggart (29) revealed a similar competitive inhibition between NMN, tetraethylammonium, and other compounds [Farah, Frazer & Porter (43)].

The rate of renal excretion of a number of "weak" organic bases has been shown to vary inversely with the pH of urine (117). For these substances the tubular transport is effected by passive "non-ionic diffusion", the cell membranes being better permeable to the un-ionized form than to the ionized. If tubular fluid is acid, there results a concentration gradient for the un-

ionized form, permitting a passive diffusion from blood or interstitial fluid to urine. The rate of transport and the direction of transport are dependent on the pK of the substances concerned. Some (quinacrine, procaine) are secreted by the tubules, when the urine is acid, but reabsorbed when the urine is rendered alkaline. The clearance of others (quinine) also varies inversely with urine pH, but never exceeds filtration rate. Whether the excretion rate of the strong bases, whose active transport has been demonstrated, also depends on urinary pH to some degree, cannot be ascertained for lack of experimental evidence.

*p-Aminohippurate (PAH).*—The clearance of PAH continues to be a popular and reliable measure for the estimation of renal plasma flow. The renal extraction ( $E_{PAH}$ ) seems to be very constant around 0.9 in man. Even at diminished clearances the extraction seems to show little deviation [Reubi (127)], a very important prerequisite for the clinical use of the method.

In the dog, apparently, the extraction of PAH is less consistent, scattering between 0.61 and 0.96. Harth, Kreienberg & Lutz (59), in an attempt to evaluate some of the factors which might influence the extraction of PAH in this species, found that in individual dogs there is an inverse relation between renal plasma flow and  $E_{PAH}$ . Furthermore, the  $E_{PAH}$  is diminished at decreased hematocrit values produced by the infusion of Tyrode solution.

Bergström, Bucht & Josephson (14) determined the true renal blood flow by means of  $^{131}\text{I}$  iodopyracet (Diodrast) and renal vein catheterization. Since several pitfalls of the usual methods can be avoided—for example, by analysis of whole blood instead of plasma—the method is claimed to give more reliable results. Bergström *et al.* (15), examining the renal extraction of PAH in patients with kidney diseases, paid special attention to the “depression limit”, i.e., the lowest plasma PAH-concentration at which the extraction ratio started to decrease. This concentration was quite normal, not only in healthy subjects but also in those patients in whom the renal extraction of PAH was diminished by the passage of part of the blood through inactive tissue (scars, tumors). In cases in which the transport activity of the tubular cells was supposed to be diminished, the depression limit was found to be lower than normal (pyelonephritis, acute glomerulonephritis).

*Urea.*—The mechanism of urea excretion continues to be investigated—and to remain controversial. Since the statement of Ullrich & Jarausch (169) that in dogs the urea concentration in tissue water at the tip of the papilla is always nearly the same as in the urine, it is generally believed that the collecting ducts are highly permeable to urea. Others, such as Schmidt-Nielsen (141) and Levinsky & Berliner (86), found the urea concentration in papillary tissue somewhat lower than in urine, which led them to believe that at constant urine flows the urea is brought to the papilla by passive diffusion out of the collecting ducts. During rising urine flows, however, when the urine urea concentration tends to fall, the papillary urea pool is washed out into the urine, leading to the well-known phenomenon of increased urea clearance or exaltation. In the sheep on a low-protein diet, the urine urea

concentration is very low (around 5 times the plasma urea concentration as compared to up to 300 times at a normal-protein diet) and independent of urine flow [Schmidt-Nielsen *et al.* (143)]. These low-protein sheep unexpectedly show a very marked exaltation phenomenon at rising urine flows. Schmidt-Nielsen & O'Dell (142) extended these studies by analysing the urea content of kidneys of sheep on low- and normal-protein diets. It was shown that, whereas on a normal-protein diet the urea concentration increases or remains constant along the axis of the renal medulla, it shows a maximum in the inner stripe of the outer medullary zone and decreases towards the papilla in sheep with low-protein diets. This different mode of urea excretion is independent of plasma urea concentration and glomerular filtration rate. It is suggested that the accumulation in the outer zone of the medulla could take place through an active reabsorption of urea in the thick ascending limb. Active urea transport was equally invoked by Crawford, Doyle & Probst (28) to explain the phenomenon that urea may reduce the water requirement for the excretion of nonurea solutes in the rat.

Clearance studies on different alkyl-thioureas by Bergmann *et al.* (13) are highly suggestive that these substances are transported passively across the tubular wall, since some of the excretion characteristics are a linear function of the number of carbon atoms attached to the nitrogen atoms of thiourea.

*Uric acid.*—By the infusion of uric acid in man undergoing mannitol diuresis, Gutman, Yü & Berger (57) found instances of uric acid clearances higher than glomerular filtration rate. By the use of the uricosuric agent sulfoxypyrazolidine, the urate clearance was further elevated. The results support the hypothesis that uric acid excretion in man involves glomerular filtration, tubular reabsorption, and tubular secretion.

It had been generally assumed that the mechanism of uric acid excretion was not disturbed in gouty subjects. Since previous clearance studies had been performed at markedly different plasma urate levels in gouty and non-gouty subjects, Nugent & Tyler (107) compared the excretory mechanisms of uric acid in gouty subjects to that in normal subjects in which the blood uric acid concentration had been elevated by the application of uric acid or its precursors. If studied at comparable plasma uric acid levels, the normal subjects reabsorbed a smaller proportion of filtered uric acid than the gouty patients. The authors conclude that abnormal renal excretion of uric acid is one important cause of hyperuricemia in some gouty patients.

In the chicken, net tubular secretion accounts for some 80 per cent of total urinary urate [Berger, Yü & Gutman (10)]. As should be expected from this fact, most compounds which have a uricosuric effect in man reduce the urate excretion in the chicken. These include probenecid, sulfoxypyrazolidine, zoxazolamine, and high dosages of phenylbutazone. Pyrazinamide and sodium *r*-lactate, substances which reduce the excretion of urate in man, had no effect on the urate excretion in the chicken.

In rabbit kidney slices, uric acid at  $7 \times 10^{-4}$  M inhibited the accumulation

of PAH *in vitro*, but no indication of active accumulation of urate by the slices could be found [Despoupoulos (37)].

#### MISCELLANEOUS

Sulfate and thiosulfate mutually inhibit each other's reabsorption by a competitive mechanism in the dog [Berglund, Helander & Howe (12)]. Apart from glomerular filtration, sulfate is reabsorbed and thiosulfate is both reabsorbed and secreted by the tubuli. Carinamide blocks the tubular secretion of thiosulfate but apparently does not affect the reabsorption of either sulfate or thiosulfate. On the other hand, sulfate seems to block both the reabsorption and the secretion of thiosulfate. The reabsorption of sulfate is practically not blocked by sodium ferrocyanide or sodium bicarbonate [Berglund (11)]. This is consistent with the assumption that in order to inhibit the reabsorption of sulfate the anions must be reabsorbed as such (thiosulfate, acetate, nitrate, phosphate), and supports the view that bicarbonate is reabsorbed secondarily to  $H^+$  secretion as  $CO_2$  and water and not in the ionic form.

By the use of an "inositolless" mutant of *Neurospora crassa*, Perlès, Colas & Blayo (115) studied the clearance of inositol in the dog. Under normal conditions this clearance is very low, indicating an almost total tubular reabsorption. With intravenous infusion of inositol, the clearance rises, approaching creatinine clearance. The maximum reabsorption was dependent on glomerular filtration rate. From studies on diabetic subjects, Colas, Perlès & Malangeau (26) conclude that the reabsorption of inositol is inhibited by saturation of the transport mechanism with glucose.

#### VOLUME REGULATION AND ALDOSTERONE RELEASE

*Regulation of aldosterone release.*—Two reviews pertinent to the regulation of aldosterone release were published in the *Proceedings of the Laurentian Hormone Conference* of 1958 by Farrell (44) and by Bartter *et al.* (6).

Farrell's work was mainly concerned with the central integration which he located at the brainstem level, mainly the central gray substance of the rostral midbrain, which seems to cause release of a tropic factor arising from the posterior commissuropineal area, which in turn acts on the zona glomerulosa of the adrenal cortex. Further, he presents new evidence to emphasize the importance of right atrial distention in depressing the aldosterone output in anesthetized dogs (1). It should be remembered that Farrell (45) maintained that the secretion of aldosterone is controlled by a cerebral, probably diencephalic, structure and that the transmission to the adrenal cortex is mediated by a humoral factor. In his recent review (44) further evidence is submitted to support this statement.

The previous statement of Farrell, that, if the level of blood electrolytes alters the secretory rate of aldosterone at the adrenal level, this effect is probably of minor importance compared to other physiological mechanisms, has gained further support.



Newman, Redgate & Farrell (105) had shown that in the cat the most effective lesions reducing the output of aldosterone were those involving the posterior diencephalon and rostral midbrain. Later studies strongly suggest that the central gray substance of the vicinity of the cerebral aqueduct contains elements important in regulating aldosterone production. As to the effector mechanism for aldosterone secretion, Farrell (45a) has strong reasons to postulate the existence of a hormone, different from ACTH, for which he suggests the name adrenoglomerulotropin or GTH. Since there is no doubt that ACTH stimulates the aldosterone production to some degree [Ross *et al.* (129)], any biological assay procedure for GTH activity must include a test for cortisol or glucocorticoid activation.

Brain extracts of beef posterior diencephalon show the highest activity in stimulating aldosterone secretion and at the same time are without effect on cortisol output. This same area of the brainstem is pointed out by lesion studies in the cat as being important for the regulation of aldosterone output.

Further evidence to support the assumption of a humoral factor influencing aldosterone secretion is provided by the cross-circulation experiments of Yankopoulos and co-workers (31, 189). Cross circulation was established through the femoral vessels or isolated adrenals of normal animals with blood from hyperaldosteronemic dogs.

Bartter *et al.* (6) gave a full description of the work which the NIH group has done towards explaining the control and action of aldosterone, including an outline of the papers of Mills, Casper & Bartter (100), and of Bartter, Mills & Gann (7). They reviewed the puzzling situation which arises from the existence of two different kinds of receptors for augmenting and diminishing the output of aldosterone. Constriction of the inferior vena cava above the liver or constriction of the common carotid artery low in the neck both result in an increase of aldosterone output as measured in adrenal venous blood. This rise is not prevented by vagal section but fails to occur after denervation of the carotid artery. The release of the caval constriction on the other hand causes a fall of the aldosterone output to normal if the vagi are intact but not after vagotomy. It is concluded that the stimuli for the increase of aldosterone production are mediated by pathways different from those which lead to a decrease, only the latter requiring the vagi to be intact. Since vagal section alone does not affect the aldosterone secretion, it is unlikely that the normal rate of aldosterone output is regulated by a tonic inhibition mediated by the vagi. This is further supported by the findings of Davis, Yankopoulos & Holman (32) that vagal section does not prevent the hyperaldosteronism and sodium retention found in the dog after the constriction of the inferior vena cava. That section of the carotid sinus nerves and the aortic depressor nerves also failed to inhibit hyperaldosteronism is not strictly a contradiction to the statements of Bartter *et al.*, since these authors only stated that this procedure inhibited the aldosterone-depressing effect elicited by common carotid occlusion and not by caval constriction. The findings of Davis *et al.* seem to indicate, however, that the

carotid sinus and aortic depressor nerves do not represent the only pathways mediating a reduction of aldosterone output.

The experiments of Denton, Goding & Wright (36) deserve special mention since they were all done in conscious, trained, and confident animals. This is extremely important since surgical procedures as well as emotional stimuli in themselves tend to stimulate aldosterone secretion (45), and increases over an initially high level may be difficult to demonstrate (136). Merino sheep were used throughout. In most instances, one adrenal gland was transplanted into a skin loop containing the carotid artery and jugular vein (94), the other adrenal gland was removed later. After the application of cuffs around the loop cranial and caudal to the graft, a needle can be inserted into the carotid artery to alter the adrenal arterial blood, or into the jugular vein to drain off and collect the adrenal venous effluent. Even a transient "adrenalectomy" may be performed without anesthesia or undue stress. A chronic unilateral parotid fistula provided both a convenient means for sodium depletion (33 to 35), since the animal loses 2 to 4 liters of saliva per day containing 180 m.eq. per liter of sodium, and a reliable test for aldosterone activity, since the sodium-to-potassium ratio of saliva drops from around 15 to below 1 in the sodium-depleted state as well as after an appropriate dose of aldosterone. "Adrenalectomy" during the state of sodium depletion promptly causes the salivary sodium-to-potassium ratio to rise again.

A local lowering of sodium and elevation of potassium content of adrenal arterial blood failed to elicit an acute response in the parotid, nor did a local elevation of the sodium content during the state of sodium depletion reproduce the effect of rapid correction of the sodium deficit made by systemic sodium application. However, the local reduction of sodium and increase of potassium concentration had some limited effect in stimulating the electrolyte active steroid production if continued long enough, or in preventing the inhibition of it during rapid systemic sodium depletion. It is reasonable therefore to assume that changes of the ionic environment of the adrenal may be a contributory cause of changing aldosterone output, but do not account for the whole range.

Cross-circulation experiments feeding the blood of the donor back to its own carotid artery after passing an "isolated" (cuffs!) adrenal graft of a recipient strongly suggest that the adrenal gland is affected by a humoral stimulus other than ionic change and most probably other than ACTH.

It cannot be overemphasized that aldosterone represents only one out of several factors influencing the sodium reabsorption by the renal tubules. Expansion of plasma volume by the infusion of plasma or iso-osmotic albumin preparations results in a natriuresis and diuresis in the anesthetized dog [Atkins & Pearce (2)], without significant changes in creatinine clearance, although the *p*-aminohippurate clearance was usually increased. Bilateral cervical vagotomy sometimes decreased but never abolished the effect. Possibly the changes in renal medullary circulation reported by Kramer *et al.*

(77, 162) may be partly responsible for the effect. But the subject is still controversial.

Bergström and co-workers (16) failed to detect any consistent alteration of glomerular filtration rate urine volume, or sodium, potassium, and chloride excretion on expansion of the plasma volume by dextran in man, provided the salt-water ratio of the body fluids was not changed. However, since the period of observation was extended for only 50 minutes after the onset of the infusion, the time was probably too short to detect any alteration in aldosterone activity.

Keeler (72) caused a striking increase in urinary sodium output by destruction of the area of the paraventricular nuclei in rats. The natriuresis started immediately after the operation and lasted for about 12 hours. Denervation of the kidneys or adrenalectomy did not affect the phenomenon, while after hypophysectomy the effect was diminished though not abolished. The meaning of these experiments is not clear. A possible explanation might be sought in the acute release of oxytocin.

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